EFFECT OF STATIN THERAPY ON SERUM LIPASE AND BLOOD GLUCOSE LEVELS IN PATIENTS WITH HYPERLIPIDEMIA

Dissertation

Submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfilment of the requirements for the award of the degree of

M.D. PHARMACOLOGY

Branch VI

April 2016

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CERTIFICATE

This is to certify that this dissertation entitled "**Effect of Statin Therapy on Serum Lipase and Blood Glucose Levels in Patients with Hyperlipidemia"** is a bonafide record of the work done by **Dr. Prathab Asir. A** under my guidance and supervision in the Department of Pharmacology during the period of his postgraduate study for **M.D. Pharmacology [Branch – VI]** from 2013-2016.

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DECLARATION

I, Dr. Prathab Asir. A here by submit the dissertation titled **"Effect Of Statin Therapy on Serum Lipase and Blood Glucose Levels in Patients with Hyperlipidemia"** done in partial fulfillment for the award of the degree M.D. Pharmacology [Branch – VI] in Sree Mookambika Institute of Medical Sciences, Kulasekharam. This is an original work done by me under the guidance and supervision of **Dr. Rema Menon. N.**

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I would like to express my gratitude to my professor and Head of Department of Pharmacology, my mentor and guide **Dr. Rema Menon. N,** for her valuable and constant guidance, supervision and support to endure the hardship throughout the study. Her patience and understanding during the times of difficulties in the study period helped me a lot to overcome the circumstances. Her constant motivation has helped me to overcome all the challenges and difficulties that I came across during this research work. Her encouragement from the initiation of this research to its culmination has always been profound. It has been an extraordinary experience working under her.

I am very much grateful and thankful to my Co-Guide **Dr. Kaniraj Peter. J,** Professor and Head, Department of General Medicine for his valuable support and guidance all throughout the study. **Dr. Madhavrao. C,** Assistant Professor of Pharmacology had always been a source of inspiration and had been of immense help with his extensive ideas, valuable contributions, patience and generous encouragement during the research work and preparation of the manuscript.

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I extend my sincere heartfelt thanks to **Dr. Velayuthan Nair,** Chairman and **Dr. Rema V. Nair,** Director, for permitting me to carry out the study in the hospital and providing facilities to accomplish my dissertation work. Then I would also like to thank the Principal of the Institution **Dr. Padmakumar** for his valuable support extended to me.

I express my special thanks to my colleague **Dr. Anandhalakshmi. A,** for giving me uncountable constructive ideas and encouragement to do the study. I also thank my senior Post Graduates **Dr. Biacin Babu, Dr. Parvathy. R.L, Dr. Navaneeth. A** and **Dr. Shanthi. M** and my junior Post Graduates **Dr. Arjun . G . Nair, Dr. Sushmita Ann. S. J.,** and **Dr. Suhaina. A** for their help and support.

Last but not the least, I thank **Mrs. Florence Vimala. P** and **Ms. Sangeetha. M** for the assistance and help extended to me during the study.

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Effect of Statin Therapy on Serum Lipase and Blood Glucose Levels in Patients with Hyperlipidemia

Abstract

Background:

Hyperlipidemia is an abnormality in the lipoprotein metabolism that leads to alterations in the various lipoproteins and the prevalence was 37.5% in age group of 15 - 64 years. Hypercholesterolemia was identified to be the risk factor for coronary artery disease and cerobrovascular disease. Statins are the hypolipidemic drugs which helps in lowering the blood cholesterol and lipoprotein levels that acts by inhibiting the 3-Hydroxy-3-methyl glutaryl coenzyme A [HMG-CoA] reductase enzyme. Apart from various adverse effects mentioned in textbooks, there were case reports mentioning that statins can be a cause for the development of acute pancreatitis [AP], where there was an elevation in the levels of serum lipase, serum amylase and blood glucose. Hence it was hypothesized in this present study that, statin therapy in hyperlipidemic patients increases the levels of serum lipase and blood glucose.

Aims and objectives:

To evaluate the effect of statin therapy on serum lipase and blood glucose levels in hyperlipidemic patients.

Materials and methods:

This study included a total number of 71 participants who were diagnosed newly as hyperlipidemic and was advised as to start either on atorvastatin or rosuvastatin. The participants were explained about the study and consent was

obtained from each participant for collection of 3 ml of blood for the estimation of serum lipase and blood glucose levels. The participants were advised to review after 3 months of therapy for estimation of serum lipase and blood glucose estimation. The study parameters are expressed in mean±Standard deviation. The $P<0.05$ was considered statistically significant.

Results:

There was a significant increase in the serum lipase [$P<0.0001$] and blood glucose [$P<0.0005$] levels, after 3 months of statin therapy in hyperlipidemic patients.

Conclusion:

Three months of treatment with atorvastatin or rosuvastatin significantly increases the levels of serum lipase and blood glucose when compared with the base line.

Key words: Pancreatitis, hyperglycemia, hyperlipasemia, blood glucose, serum lipase, hypercholesterolemia, statins, HMG-CoA reductase inhibitors.

1. Introduction

Abnormalities in the metabolism of lipoprotein can lead to hyperlipoproteinemia which causes alterations in the various lipoproteins.[1] The prevalence of dyslipidemia in adult in the age group of 15 - 64 years was 37.5 % as reported by the Indian Council of Medical Research [ICMR] surveillance project.[2] An epidemiological study have proved that there is a strong relationship between serum cholesterol levels and premature coronary artery disease [CAD].[2] Hypercholesterolemia has been identified as a risk factor for CAD[3] and cerobrovascular disease [CVD][4] and the need for the intervention was established by the Framingham Heart Study in 1949 and 1961.[3] CAD is the commonest cause for death and 25 % of the death that occurs in India was due to CAD in the year 1990.[3] Hyperlipidemia is the most common cause of artherosclerosis of the blood vessel and these changes in the arteries to the central nervous system leads to CVD like strokes and transient cerebral ischaemia.[5]

Statins are a group of hypolipidemic drugs which act by inhibiting the 3-Hydroxy-3-methyl glutaryl coenzyme A [HMG-CoA] reductase enzyme.[6] These drugs help in lowering the blood cholesterol and lipoprotein levels. The use of hypolipidemic drugs has shown to reduce the atherosclerotic changes and thereby prevent cardiovascular disease in patients with hyperlipidemia.[7]

Lovastatin, fluvastatin, mevastatin, simvastatin, pravastatin, atorvastatin, rosuvastatin and pitavastatin are in HMG-CoA reductase inhibitors.[6,7]

Even though statins are well tolerated, they produce adverse drug reactions [ADRs] which includes myalgia, gastrointestinal [GI] disturbances, raised liver enzymes, insomnia and rash. More serious and rare adverse effects are rhabdomyolysis and angio-oedema.[8]

There were case reports published around the globe stating and confirming that statins can be a cause for the development of acute pancreatitis [AP] where there is elevation of serum lipase, serum amylase and blood glucose level.[9-14]

The hypothesis of present study is that statin therapy in hyperlipidemic patients increases serum lipase and blood glucose levels. Based on case reports on statin induced pancreatitis[9-14] it is assumed that there may be an impact on the pancreas which can lead to subclinical elevation in the levels of serum lipase and alteration in the blood glucose levels.

Considering the burden, severity and mortality due to AP, an extensive review of literature showed that there were no similar studies done on the effect of statins on serum lipase and blood glucose levels in South India. Hence it was planned to evaluate the effect of statin therapy on serum lipase and blood glucose levels in hyperlipidemic patients.

2. Aims and Objectives:

To evaluate the effect of statin therapy on serum lipase and blood glucose levels in hyperlipidemic patients.

REVIEW OF LITERATURE

3. Review of Literature

3.1. Lipids:

Lipids are biochemically important heterogeneous compounds that are water insoluble but are soluble in non-polar organic solvents.[15] They are transported in plasma in lipoproteins which are complex larger macromolecules. Lipoproteins play a major role in the dietary cholesterol absorption.[1] These lipoproteins can be visualized only by electron microscopy and separated by using ultracentrifugation.[16]

3.1.1. Classification of lipoproteins:

They are classified depending upon the size, density and composition of the lipoproteins.[17]

a) Chylomicrons
b) Very low-density lipoproteins [VLDLs]
c) Intermediate density lipoproteins [IDLs]
d) Low-density lipoproteins [LDLs]
e) High density lipoproteins [HDLs]

3.1.1.1. Chylomicrons:

Chylomicrons are less dense lipoprotein with a density of <0.95g/ml and the largest among the lipoprotein. The proportion of various proteins in this includes apo B-48, apo A-I, apo A-II, apo A-IV, apo C-II and apo E. The S_f of more than 400 is considered as the largest

and S_f of 20-400 is designated as smaller chylomicrons. The chylomicron core predominantly consists of triglycerides and originates from the diet.[16,17]

Chylomicrons are synthesized and formed in the small intestine and then transported to the blood stream by lymphatics through the left subclavian vein.

3.1.1.2. Very low density lipoproteins [VLDL]:

They are the largest lipoproteins which contain lipids that are endogenously produced with the density ranging from 0.95 to 1.006 g/mL. The major component of VLDL is apo B-100 and it also includes apo C-I, apo C-II, apo C-III, apo E and apo A.[17]

These are transported in the blood from the liver to the muscle and adipose tissue, where apo C-II activates the lipoprotein lipase leading to release of free fatty acids from VLDL.[16]

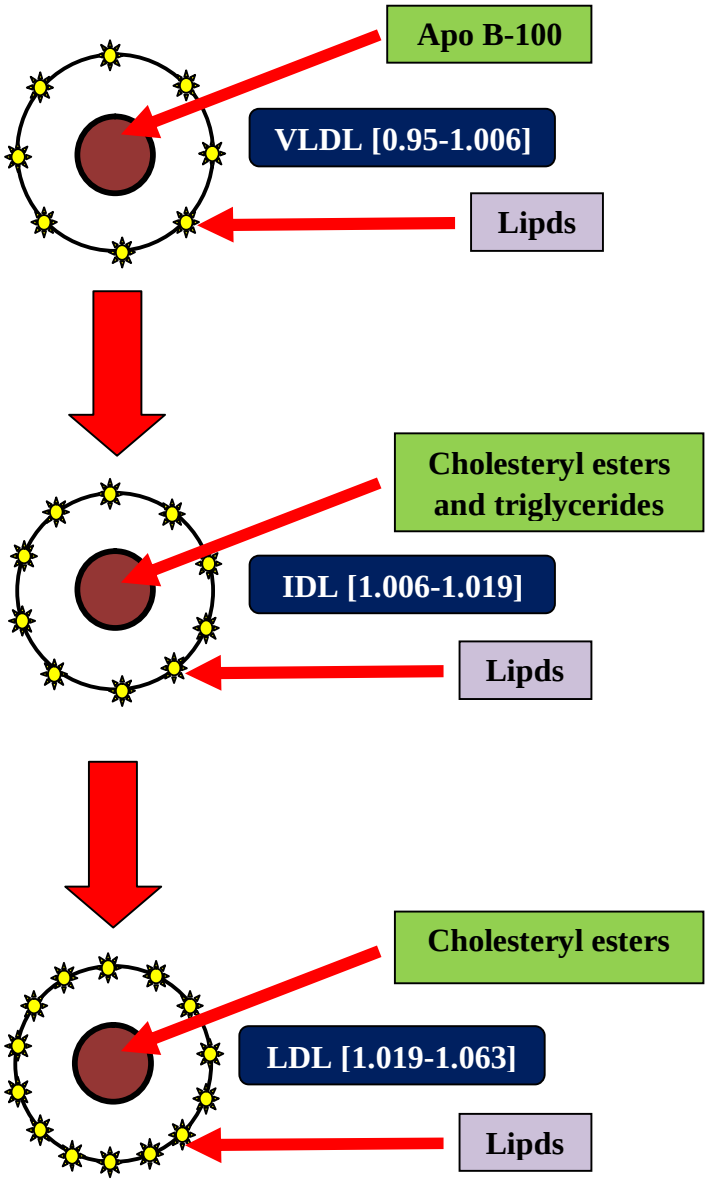
3.1.1.3. Intermediate density lipoproteins [IDL]:[17]

The density of IDL ranges from 1.006 to 1.019 g/ml and are formed during the process of formation of low-density lipoprotein from very low-density lipoprotein. The intermediate-density lipoprotein core consists of cholesteryl esters and triglycerides.

3.1.1.4. Low density lipoprotein [LDL]:[17]

Low density lipoproteins have cholesterol as the major part with the density ranging from 1.019 to 1.063 g/ml which contains lipoproteins and is the product that is formed at the end of the catabolism of very low density lipoprotein. The main protein component is apo B-100 and the low density lipoprotein core is formed of cholesteryl esters.

Figure 1. Showing the density and formation of various lipoproteins by catabolism



3.1.1.5. High density lipoprotein [HDL]:

High density lipoprotein are smallest and the most dense lipoprotein lying between the range 1.063 to 1.210 g/mL.[17] High density lipoproteins are rich in protein and is formed from the liver and small intestine.[16]

They contain apo A-I alone, both apo A-I and apo A-II together or apo A-II alone, apo C-I, apo C-II and other apolipoproteins as well as lecithin cholesterol acyl transferase [LCAT] enzymes.[16,17]

High density lipoproteins are sub classified depending upon the density and size as HDL_2 and HDL_3 and latter are smaller and denser than former.[17]

During electrophoresis the alpha region attracts both HDL_2 and HDL_3. Lipid poor high density lipoproteins which are discoidal in shape migrates to the pre-β region during electrophoresis and they are also known as pre- β_1 High density lipoprotein.[17] These discoidal shaped pre-β_1 HDL particles acquire cholesterol and forms a larger particle called as pre-β_2 HDLs and is preferably the substrate for lecithin cholesterol acyltransferase.[17]

3.1.2. Synthesis of cholesterol:[18]

Cholesterol is a C_{27} steroid and is synthesized from acetyl coenzyme [CoA]. The first stage of cholesterol biosynthesis starts from three acetyl

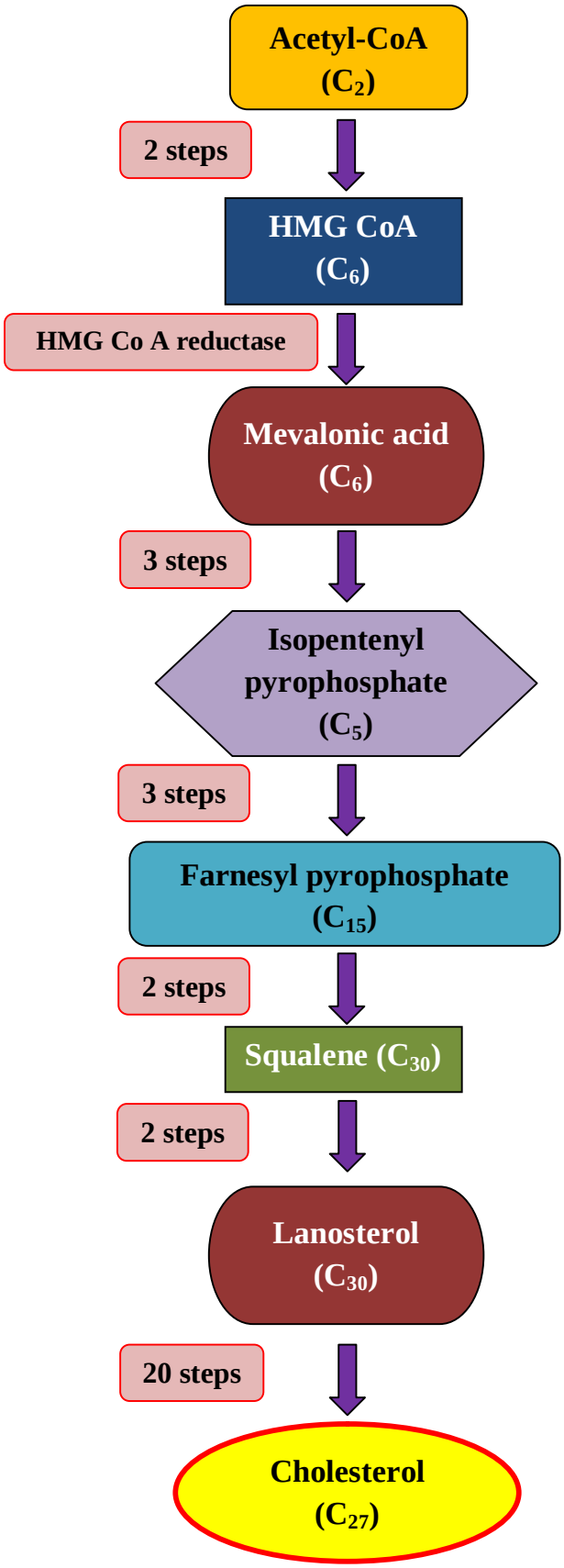
CoA to form 3-hydroxy-3-methylglutaryl Coenzyme A. The conversion of 3-hydroxy-3-methylglutaryl Coenzyme A to Mevalonic acid happens by reducing the thioester of 3-hydroxy-3-methylglutaryl Coenzyme A to primary hydroxyl group in the presence of 3-hydroxy-3-methylglutaryl Coenzyme A reductase enzyme which is the primary rate controlling enzyme in cholesterol synthesis. Mevalonic acid is then converted to isopentenyl pyrophosphate by successive phosphorylation and decarboxylation.

The second stage of cholesterol biosynthesis is coupling of six molecules of isopentenyl pyrophosphate, of which three molecule of isopentenyl pyrophosphate condense to form a farnesyl pyrophosphate. Two molecule of farnesyl pyrophosphate thus formed forms a single squalene. The squalene thus formed initially undergoes epoxidation and cyclization to form lanosterol.

The final stage is the formation of cholesterol from lanosterol by removal of three methyl group from lanosterol causing reduction of the side chain double bond and movement of other double bond within the ring structure.

The cholesterol biosynthesis is schematically presented in Figure 2.

Figure 2. Cholesterol Biosynthesis



3.1.3. Hyperlipidemia:

Abnormalities in the metabolism of lipoprotein can lead to hyperlipidemia which causes alterations in the various lipoproteins.[1] Dyslipidemia is an important factor that predispose to early cerebrovascular disease [CVD] and coronary artery disease [CAD].[19]

According to the National Commission on Macroeconomics and Health [NCMH] there will be approximately 62 million patients with coronary artery disease by 2015 and of these 23 million would be below the age group of 40 years.[20] World Health Organization report 2002, has predicted that death due to coronary artery disease in India will be 2.6 million by 2020.[21]

Hyperlipidemias are classified as primary and secondary hyperlipidemias. Primary hyperlipidemia includes hypercholesterolemia and hypertriglyceridemia and is due to an inherent genetic defect of lipid-lipoprotein-apoprotein metabolism.

Secondary hyperlipidemia includes hypercholesterolemia and hypertriglyceridemia which are caused due to other underlying causes.[22] According to Indian Council of Medical Research [ICMR] surveillance project the prevalence of dyslipidemia among young male workers in industry was estimated to be 62 % whereas it was 37.5 % among the other population of age 15 - 64 years.[2]

3.1.3.1. Hypercholesterolemia:[19]

Hypercholesterolemia is defined as the increased level of LDL and decrease in the HDL level and is associated with higher risk for development of morbidity and mortality due to CAD and CVD.

3.1.3.2. Hypertriglyceridemia:

Hypertriglyceridemia is a condition in which there will be an elevation in the triglyceride level and is associated with hyperlipidemias, type 2 diabetes mellitus [DM] and metabolic syndrome.[23] Patients suffering from hypertriglyceridemia are often obese, hypertensive, insulin resistant or diabetic[23] and are at high risk of developing CAD[24] and acute pancreatitis.[25]

3.1.4. Causes of hyperlipidemia:

The underlying factors that lead to primary hyperlipidemia are genetic defects like polygenic hypercholesterolemia, decreased LDL clearance, primary clearance defect combined with secondary excess production of Triglyceride [TGL], familial combined hyperlipidemia, familial monogenic hypercholesterolaemia, familial deficiency of lipoprotein lipase [LPL], familial deficiency of apoprotein C_{11}, familial endogenous hypertriglyceridemia and familial type V hyperlipoproteinaemia.[22]

The factors that cause secondary hyperlipidemia are hypothyroidism, hypopituitarism, diabetes mellitus [DM], glycogen storage disease, anorexia nervosa, bulimia, obesity, diets rich in saturated fat and carbohydrate,

excessive alcohol consumption, Cushing's syndrome, nephrotic syndrome, cholestasis, acute intermittent porphyria, dysglobulinaemia, pancreatitis, chronic renal failure, chronic liver disease, intake of drugs like beta blockers, diuretics, glucocorticoids, oestrogens, oral contraceptive pill [OCP], or disease like systemic lupus erythromatosis [SLE] and pregnancy.[22]

Study conducted by García-Unciti et al.[26] had revealed that life style changes like diet modification and exercise in hyperlipidemic patients had significantly decreased the weight and waist circumference as well as the serum levels of low-density lipoprotein [LDL] and total cholesterol [TCH].

De Goeij et al.[27] in their study identified that there was an association between abnormal lipid levels and worsening of renal function in patients suffering from chronic kidney disease [CKD].

3.1.5. Normal lipid levels:[28]

The third report of the National Cholesterol Education Program [NCEP] Adult Treatment Panel III [ATP III] on detection, evaluation, and treatment of high blood cholesterol in adults by the expert panel states that LDL cholesterol will be the primary target for therapy.

According to the ATP III guidelines, all adults above the age of 20 years should undergo evaluation of lipid profile once in 5 years. The normal value of lipid profile as suggested by National Cholesterol Education Program Adult Treatment Panel III [NCEP ATP III] is as follows,

Table 1. Normal values of various lipoproteins[28]

PARAMETER	NORMAL VALUE [mg/dl]
Total Cholesterol	< 200
Triglycerides	< 150
Low Density Lipoprotein	< 100
High Density Lipoprotein	40 - 60

3.1.6. Management of hyperlipidemia:

Cardiovascular morbidity and mortality due to hyperlipidemia can be lowered by decreasing the LDL and TGL levels and by increasing the HDL levels.[25]

Management of hyperlipidemia focuses on both non-pharmacological[29] and pharmacological approach.[30] Non-pharmacological approach to decrease the morbidity and mortality due to hyperlipidemia includes diet modification with low-fat and low-carbohydrate[31], physical activity for weight reduction,[29, 32] smoking cessation and reduced alcohol consumption.[29]

Kelly[31] conducted a study on the effect of aerobic exercise in hyperlipidemic patients and had proved that it increased the HDL level by 1.9 to 2.5 mg/dl on an average, decreased the TCH level by 3.9 mg/dl, LDL by 3.9 mg/dl and TGL level by 7.1 mg/dl.

Pharmacological agents for treating hyperlipidemia include statins, fibrates, nicotinic acid and bile acid sequestrants.[25,30]

In addition fish oil, plant stanols and sterols and LDL apheresis have been used to effectively modify lipid levels.[25,30]

The technique of apheresis for reducing the TGL level was first done in the year 1978 by Betteridge et al. and after that apheresis was considered as newer effective tool in the management of severe/extreme hypertriglyceridemia. Several studies had proven that management of severe/extreme hypertriglyceridemia with apheresis prevented the recurrence of acute pancreatitis and also reduced morbidity and mortality.[23]

Fares et al.[33] conducted a study with icosapent ethyl in severe hypertriglyceridemic patients and triglyceride level was found to be reduced. They also suggested that it can be used as an adjunct therapy to statins. Eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA] are the two agents that form omega-3 fatty acid,[34] where icosapent ethyl [IPE] is purest form of EPA.[33,34] Icosapent ethyl was approved by the United States Food and Drug Administration [USFDA] as an adjunct therapy along with diet in severe hypertriglyceridemic patients.[34]

A Multi-Centre, Placebo-Controlled, randomized, Double-Blind, 12-week study with an open label Extension [MARINE] and Amarin's Phase 3 Clinical Trial [ANCHOR] revealed that icosapent ethyl significantly

reduced triglyceride [TGL], non-HDL, apolipoprotein B, VLDL-C and TC levels.[34]

Ballantyne et al.[35] stated that EPA 4 g/day and 2 g/day had significantly decreased TG, non-HDL cholesterol and LDL levels. Moreover it also decreased the apolipoprotein B levels.

A pharmacokinetic study conducted by Braeckman et al.[36] concluded that atorvastatin, a substrate of CYP3A4 did not have any clinically significant interactions with warfarin, rosiglitazone and omeprazole, which were substrates of CYP2C9, CYP2C8 and CYP2C19 respectively. It was also found that approved dose of icosapent ethyl at 4g/day had no effect on the single dose of atorvastatin in healthy individuals.

Hovingh et al.[37] conducted a randomized, double-blind, placebo-controlled phase 2 trial with a new and novel cholesterol esterase transfer protein [CETP] inhibitor TA-8995 on 364 hyperlipidemic patients and have shown significant reduction in the LDL levels.

Even though various groups of drugs were used, statins are considered to be the first line drugs for the treatment of hyperlipidemia.[23]

3.2. Statins:

3.2.1. History:[6]

Endo and colleagues identified statins as an inhibitor of cholesterol synthesis which were isolated from a mould, *Penicillium citrinum* in the

year 1976. Further study conducted by Brown and Goldstein proved that statins inhibit the cholesterol synthesis by HMG-CoA reductase inhibition. Compactin was the first statin that was studied in human which was renamed later as mevastatin. The first approved statins for human use was developed by Alberts and colleagues at Merck laboratories and called as Lovastatin [formerly known as mevinolin] which was isolated from *Aspergillus terreus*. Pravastatin and simvastatin are semisynthetic where as atorvastatin, fluvastatin, rosuvastatin and pitavastatin are synthetic compounds.

3.2.2. Introduction:

Statins are the best tolerated and most effective drugs for the treatment of dyslipidemia. These drugs act by inhibiting the HMG-CoA reductase in the cholesterol biosynthesis.[6,7] These drugs are helpful in the reduction of LDL[38] and high dose of potent statins like atorvastatin, simvastatin and rosuvastatin also reduce the triglyceride level [TGL] caused by elevated VLDL levels.[6,7] All statins except atorvastatin and rosuvastatin [plasma half life 18-24 hours] are advised to be taken at night due to the peak synthesis of hepatic cholesterol by HMG-CoA reductase which has maximum activity at midnight.[6]

Statin therapy has shown to effectively reduce the cholesterol levels, which in turn reduces the mortality and morbidity due to CAD and CVD.[6]

3.2.3. Statins available:

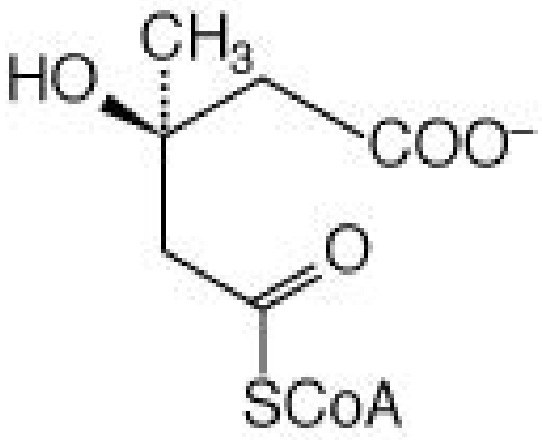
Statins currently available for the treatment of hyperlipidemia are[6-8]

- Lovastatin
- Simvastatin
- Pravastatin
- Atorvastatin
- Rosuvastatin
- Pitavastatin
- Fluvastatin

3.2.4. Structure of statins:[6]

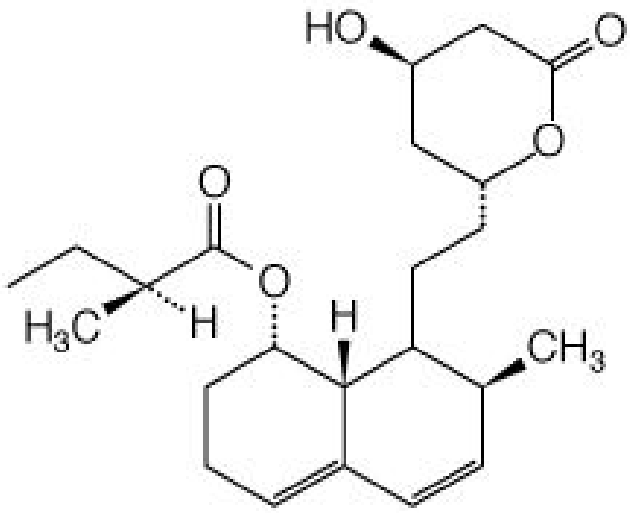
The different types of statins available vary among themselves in their pharmacokinetics, depending upon the chemical structure of individual compounds. The only common feature that all statins share is the side group which is similar to HMG-CoA.

3.2.4.1. HMG-CoA:



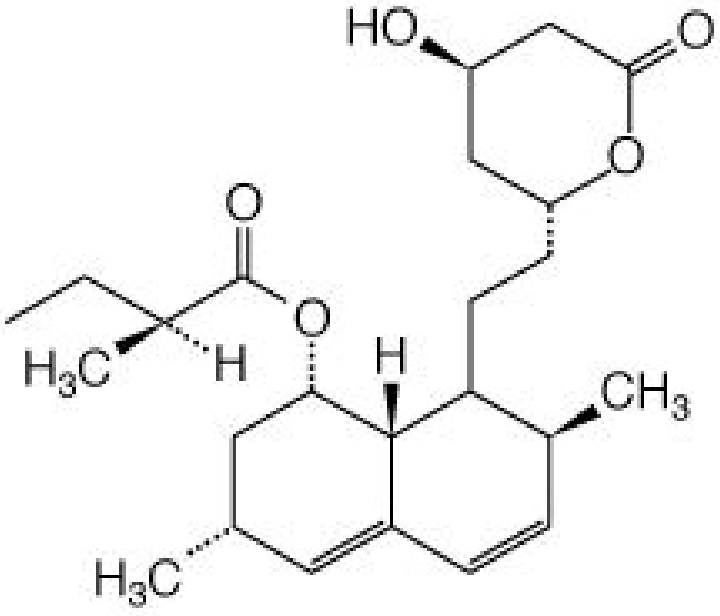
3.2.4.2. Mevastatin:

Mevastatin has a hexahydronaphthalene ring.



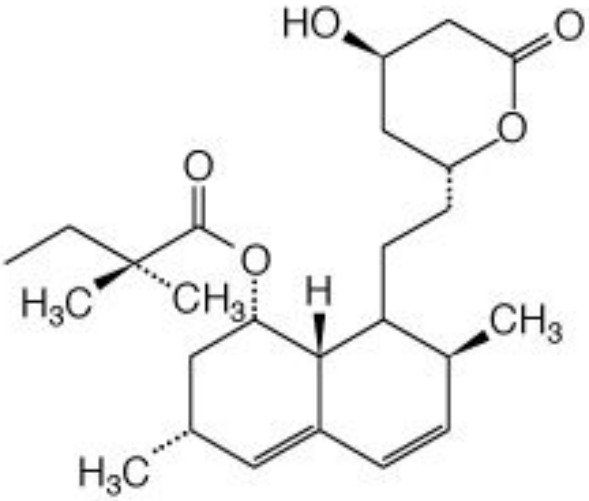
3.2.4.3. Lovastatin:

Lovastatin has a hexahydronaphthalene ring, methyl group at carbon 3 and methyl butyrate ester.



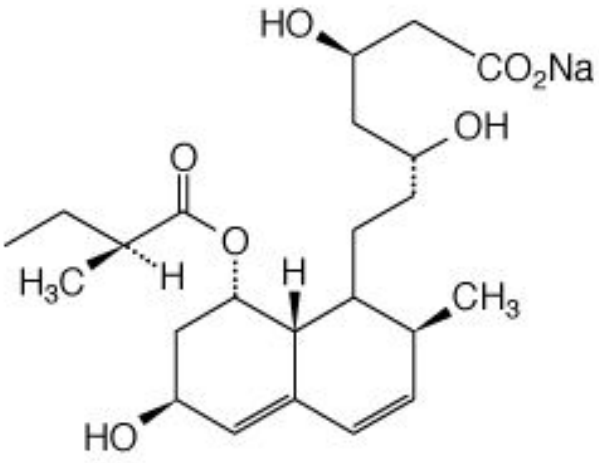
3.2.4.4. Simvastatin:

Simvastatin has a hexahydronaphthalene ring and dimethyl butyrate ester in its structure.



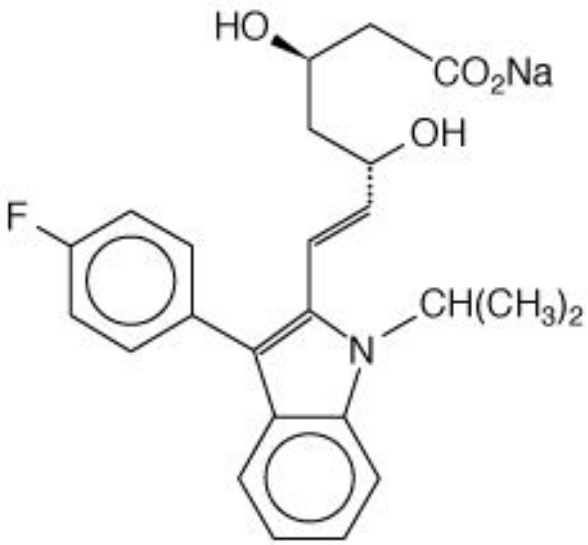
3.2.4.5. Pravastatin:

Pravastatin has a hexahydronaphthalene ring and a methyl butyrate ester in its structure.



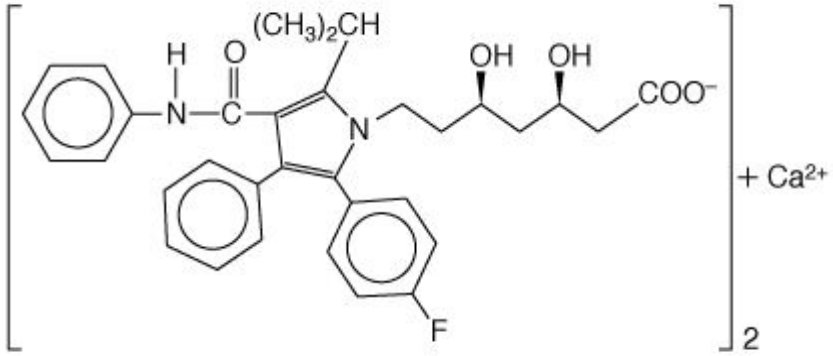
3.2.4.6. Fluvastatin:

The structure of fluvastatin has a heptanoic acid side chain



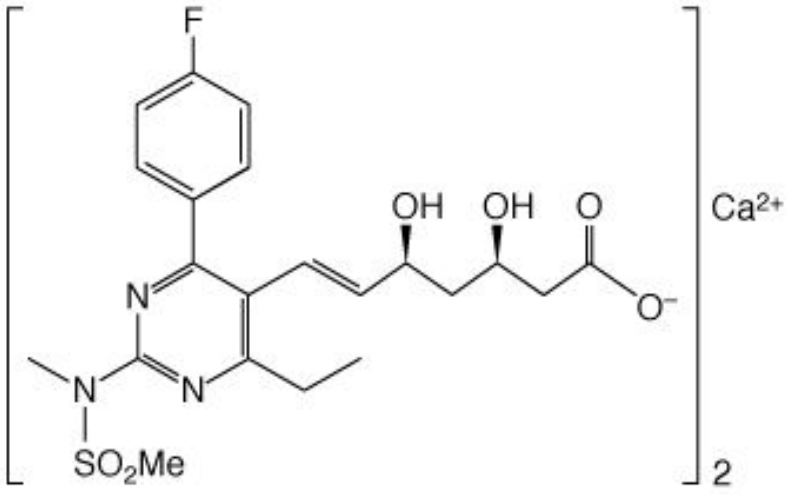
3.2.4.7. Atorvastatin:

Atorvastatin has a similar structure of fluvastatin and have a heptanoic acid side chain.



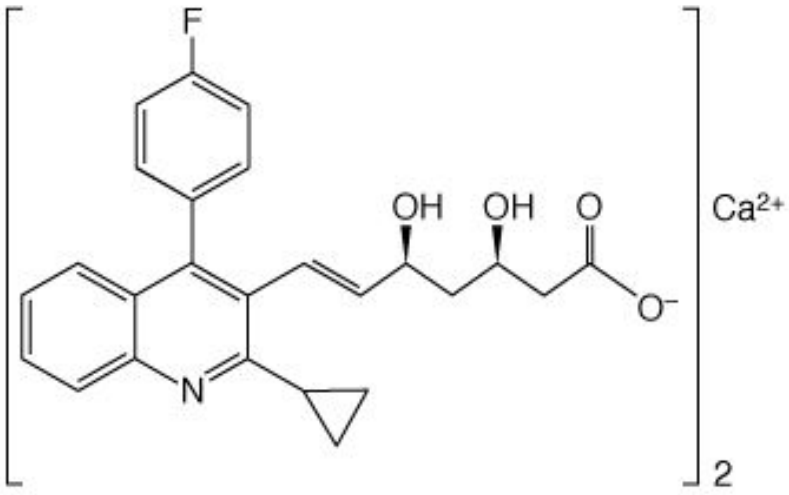
3.2.4.8. Rosuvastatin:

Rosvastatin also has similar structure to that of Fluvastatin and atorvastatin with a heptanoic acid side chain.



3.2.4.9. Pitavastatin:

Pitavastatin also has a similar structure to that of rosuvastatin with a heptanoic side chain in its structure.



3.2.5. Mechanism of action:

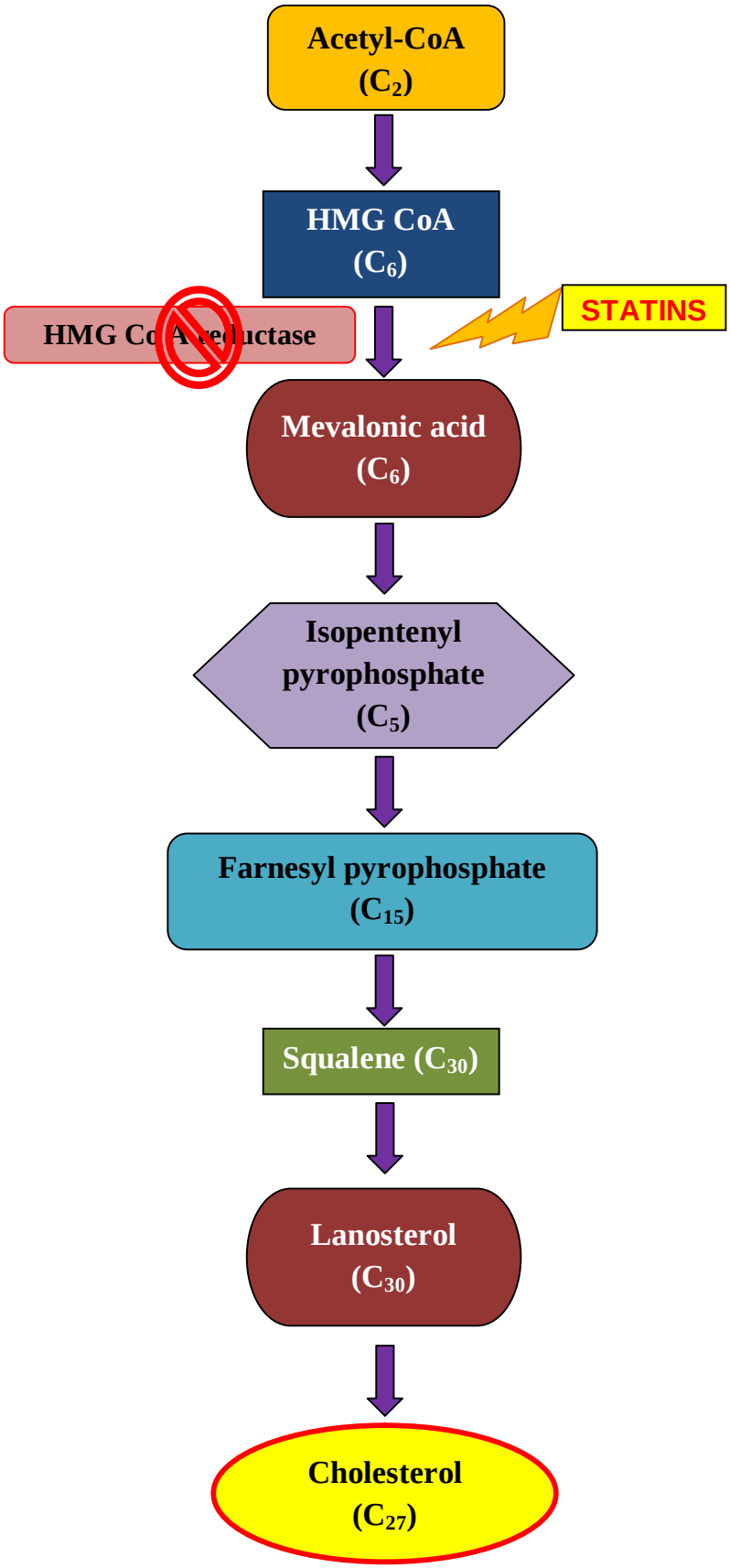
The role of statin is mainly to reduce the low density lipoprotein levels. Statins reduce the levels by preventing the formation of mevalonate. They competitively inhibit 3-hydroxy-3-methylglutaryl-coenzyme A reductase enzyme, reducing the synthesis of mevalonate from 3-hydroxy-3-methylglutaryl-coenzyme A and is the rate limiting step in the biosynthesis of cholesterol.[6]

Normally 70-75 percentage of plasma low density lipoprotein is removed by receptor mediated endocytosis by the hepatocytes. In the liver, cholesterol esters from low density lipoprotein molecules are hydrolyzed to form free cholesterol. When statins are given the *de novo* synthesis is inhibited. This leads to up-regulation of low density lipoprotein receptors on the liver cells and helps in the clearance of cholesterol rich plasma low density lipoproteins. This is dose dependent and full effect is seen within 6 weeks.[39]

Statins in their therapeutic dose can reduce the total cholesterol, triglyceride and low density lipoproteins synthesis by 20-50 %, 10-30 % and 30-55 % respectively and increase the high density lipoprotein levels by 5-15 %.[7] Higher doses of most efficacious statins like atorvastatin, rosuvastatin and simvastatin can reduce the triglyceride levels by 25-35 %.[7]

The mechanism of action of statin is schematically presented in Figure 3.

Figure 3. Mechanism of action of statins



3.2.6. Pharmacological action:

Statins reduce levels of low density lipoprotein, very low density lipoprotein and total cholesterol. Added to this, other effects which are beneficial like stabilization of plaque on the vessel wall, inhibition of thrombus formation, reduction in the viscosity of serum, anti-inflammatory and antioxidant effect are also seen.[40] These beneficial effects are due to the inhibition of vasoconstriction, activation of re-endothelialization and upregulation of endothelial nitric oxide synthase.[41]

Anti inflammatory and antioxidant effects are also seen due to the regulation of the inflammatory mediators.[41]

3.2.6.1. Antiatherogenic effects of statins:[42]

Nuclear factor kappa B plays a vital role in the initiation of inflammatory process leading to atherosclerosis, this activity was reduced by the inhibition of synthesis of farnesylated proteins by statins. They also inhibit the proliferation and migration of smooth muscle cells from vessel media into the intima and also the conversion of contractile cells to reparatory type which prevents the atherosclerotic plaque.

3.2.6.2. Effect of statins on endothelial dysfunction:[42]

Statins increase the endothelial blood flow and nitric oxide synthesis thereby improving the endothelial function.

3.2.6.3. Anti-inflammatory effects of statins:[42]

Various studies have proven that inflammation is greatly associated with CAD and atherosclerosis is dominated by immune cells. The elevation in the inflammatory markers like C- Reactive Protein [CRP], interleukin-6 [IL-6], inter cellular adhesion molecule-1 [ICAM-1] and serum amyloid A are associated with the high risk leading to initial and recurrent cardiovascular events.

Increase in the CRP levels is related to increased migration of monocyte and increased LDL uptake by macrophages. Studies have proven that there was a decrease in the levels of CRP which was greater with atorvastatin and rosuvastatin.

3.2.6.4. Antiproliferative effects of statins:[42]

Statins decrease the plaque growth by reducing the synthesis of extracellular matrix, proteins like Rac 1 and Rho A. They also inhibit the conversion of reparatory-type cells from contractile smooth muscle cells and also inhibit the migration of reparatory-type cells into the intima from the arterial media.

3.2.6.5. Effect of statin on angiogenesis:[42]

The endothelial proteinkinase Akt is activated which in turn stimulate the endothelial nitric oxide synthetase in the endothelium and neoangiogenesis. Thus stimulated protein kinase Akt improves the

metabolism of myocardial oxygen and also increases the angiopoetine level.

3.2.6.6. Effect of statin in stabilizing the plaque rupture:[42]

They decrease the activity of MMP1 and MMP3, a metalloproteinases which play the vital role in rupture of the plaque. They also reduce the arterial stiffness and rheology thereby reducing the blood pressure on long term use.

3.2.6.7. Effect of statins on thrombus formation:[42]

Statins inhibit the generation of thrombin by decreasing the activity of plasminogen activator inhibitor-1 [PAI-1], also decrease the blood fibrinolytic activity.

3.2.6.8. Effect of statins on stabilization of plaque:[42]

Even though various effects have been described for the atherosclerotic plaque stability, the important effect is due to reduction in the size of the lipid core of the plaque and prevention of the inflammatory reaction.

3.2.6.9. Effect of statin as an antioxidant:[43]

Alanazi in his study have shown that treatment with pravastatin in subjects taking primaquine can prevent oxidative damage by their antioxidant activity. It was also concluded that pravastatin can help in restoring the functions of the erythrocyte by reducing the oxidative stress.

3.2.6.10. Other findings on effects of statins:

Zhou et al.[44] conducted a meta-analysis in patients with acute coronary syndrome, and found that those who had taken statin therapy had reduced risk of atrial fibrillation.

Novaro et al.[45] in their retrospective analysis of 174 patients who were suffering from mild to moderate calcific aortic stenosis showed that there was a significant reduction in the progression of the disease.

Another randomized control trial conducted on patients planned for coronary intervention pretreated with atorvastatin 40 mg per day for one week by Pasceri et al.[46] This study showed that there was a significant reduction in the cardiac markers for myocardial damage like CK-MB, troponin I and myoglobulin.

Vyas et al.[47] who conducted a multicenteric randomized study to prove the anti arrhythmic effects of statins in patients suffering from ventricular tachycardia [VT] or ventricular fibrillation [VF] with automatic implantable cardioverter-defibrillator, suggested that statins were associated with reduction in the episodes of VT or VF.

Kuwana et al.[48] conducted a prospective, open-label, single center pilot study in patients with systemic sclerosis and concluded that statins at a dose of 10 mg per day for a period of 24 months might be helpful in treating the vascular complications of systemic sclerosis.

A retrospective cohort study conducted by Lokhandwale et al[49] on asthmatic patients who were on inhaled corticosteroids showed that statins had beneficial effects in preventing the acute exacerbation of asthma.

Multicenteric retrospective analysis of 5011 patients suffering from heart failure by evaluating the inflammatory cytokines and high-sensitivity C-reactive protein [hs-CRP] by McMurray et al.[50] had shown that there was a better outcome with rosuvastatin in patients having hs-CRP ≥2.0mg/L.

Everett et al.[51] conducted a randomized, double blinded, placebo controlled, multicenteric trial with rosuvastatin involving 17802 patients from 26 countries at 1315 sites. This study showed that there was a reduction in the incidence of stroke by 48 % and the risk of hemorrhagic stroke was not increased.

Han et al.[52] conducted a prospective, multicenteric, controlled clinical trial in China which randomized 2998 type 2 DM patients with chronic kidney disease who were planned to undergo coronary or peripheral arterial angiography, rosuvastatin 10 mg/day was administered 2 days before and 3 days after the procedure. The study outcome showed that acute kidney injury due to contrast was significantly low in rosuvastatin group and during the follow-up after 30 days showed that worsening of heart failure was also significantly reduced.

Ghosh et al.[53] conducted a study on mice and proved that pravastatin have a protective effect on the dopaminergic neurons in 1-methyl-4-phenyl-1,2,3,6-tetra hydropyridine [MPTP] induced parkinsonism.

Hsu et al.[54] in their retrospective cohort study which included 1738 blood stream infection [BSI] patients in United States of America revealed that statin use within 1 month period before BSI reduced the mortality.

Kwong et al.[55] in their cohort study stated that there was a statistically significant protection against morbidity and mortality in elderly patients with influenza.

Lee et al.[56] conducted a 1 year retrospective study on 791 patients with benign prostatic hyperplasia [BPH] and proved that statins had reduced the prostate volume and prostate specific antigen.

A nested case-control study conducted by McGwin et al.[57] at the Veterans Affairs Medical Centre in Birmingham on patients newly diagnosed to have age related maculopathy [ARM] revealed that ARM was significantly less with patients who had treatment with statin.

Harbi et al.[58] in their nested cohort study of 2 randomized controlled trials including 763 patients who were critically ill proved that statin therapy have reduced hospital mortality.

Wan et al.[59] did a meta-analysis of 5 randomized clinical trial [RCT] and 27 observational studies including 3,37,648 patients. The study revealed that RCT did not show significant reduction in the in-hospital mortality, whereas there was a significant decrease in the in-hospital mortality in the observational study.

Sathyapalan et al.[60] conducted a randomized, double blinded, placebo controlled trial with atorvastatin in patients suffering from polycystic ovary syndrome and showed that there was a significant reduction in the total cholesterol [TCH], triglycerides [TGL], free androgen index, total testosterone, serum insulin levels and homeostatic model assessment-insulin resistance [HOMA-IR].

Al-Ghoul et al.[61] conducted a study on male mice by administering simvastatin and melatonin following major thermal injury and proved that simvastatin had anti-inflammatory action.

Moraes et al.[62] conducted a study on mice with fluvastatin and simvastatin and proved that fluvastatin had a better inhibition of aggregation of platelet when compared to simvastatin.

So altogether statins have proved their effectiveness to improve coronary artery disease and cerebrovascular disease.

3.2.7. Pharmacokinetics:

3.2.7.1. Absorption:

Statins are well absorbed orally and are their absorption is enhanced by food.[63] The intestinal absorption varies between 30-85 %[6] except for fluvastatin which is absorbed almost completely.[38]

3.2.7.2. Distribution:

Pravastatin is bound 50 percent to the plasma where as all other statins are bound 95 percentage or more are only 50 % bound.[6]

3.2.7.3. Metabolism:

All statins undergo extensive first pass metabolism which is primarily mediated by organic anion-transporting polypeptide 1 B1 [OATP1B1], an organic anion transporter.[6] Except rosuvastatin, all other statins are metabolized by CYP3A4.[7]

3.2.7.4. Excretion:

After biotransformation in the liver, all statins and 70 % of its metabolites get eliminated through the faeces[6] and 5-20 % gets excreted in the urine.[38]

3.2.8. Uses:

Statins are first line drugs useful in the treatment of both primary as well as secondary hyperlipidemia associated with diabetes mellitus.[7,63] Statins can

reduce morbidity and mortality due to coronary artery disease and hence used to treat patients with myocardial infarction, angina, stroke and transient ischemic attack.[63]

3.2.9. Adverse drug reaction:

Even though statins are well tolerated, they can also cause adverse effects like headache, muscle pain, gastrointestinal disturbances, increased liver enzymes, insomnia and rash.[7,8] Other serious and rare adverse effects include rhabdomyolysis and angio-oedema.[8] Adverse effects like acute pancreatitis[9-14] and decreased libido had been reported.[64]

The effect of statin on the muscle is due to the elevated respiratory exchange ratio in asymptomatic persons, whereas in symptomatic patients there is an off-statin respiratory exchange ratio. This alteration in the respiratory function of the cell contributes to the myopathy in patients on statin therapy.[65]

Rhabdomyolysis is one of the dangerous and well recognized adverse drug reaction due to statin therapy which occurs due to severe muscle damage causing rise in the creatinine kinase levels upto 10 times the normal upper limit. This is usually associated with renal dysfunction which further leads to renal failure and even death.[65]

3.2.10. Drug interaction:

Statins are metabolized by cytochrome P [CYP] 450 isoenzymes. The isoenzyme CYP3A4 is responsible for the metabolism of atorvastatin, lovastatin

and simvastatin and CYP2C9 is responsible for the metabolism of fluvastatin and rosuvastatin.[66]

The inducers of CYP3A4 include phenytoin, phenobarbital, barbiturates, rifampin, dexamethasone, cyclophosphamide, carbamazepine, troglitazone and omeprazole; whereas they are inhibited by ketoconazole, itraconazole, fluconazole, erythromycin, clarithromycin, tricyclic antidepressants, nefazodone, venlafaxine, fluvoxamine, fluoxetine, sertraline, cyclosporine A, tacrolimus, mibefradil, diltiazem, verapamil, protease inhibitors, midazolam, corticosteroids, grapefruit juice, tamoxifen and amiodarone.[66]

The inducers of CYP2C9 includes rifampin, Phenobarbital, phenytoin and troglitazone and inhibitors include ketoconazole, fluconazole and sulfaphenazole.[66]

These inhibitors can increase the blood statin levels leading to toxicity and inducers can decrease the levels of blood statin causing therapeutic failure.[7]

3.2.11. Contraindication:

Even though there are no evidence to say that statins are contraindicated in pregnancy and lactation, they are avoided in this condition as they are not proved to be safe.[63]

Drugs which act through the CYP3A4 and CYP2C9 are also contraindicated with statin therapy as statins also act through these hepatic isoenzymes which can either get induced or inhibited by this drugs.[66]

3.2.12. Pharmacogenetics:

Lagos et al.[67] conducted a study on population of Amerindian Chile to evaluate the response of atorvastatin in patient with apolipoprotein E [APOE] polymorphisms in their variants of rs429358 and rs7412 and the low-density lipoprotein receptor [LDLR] gene 1959C>T single nucleotide polymorphism [SNP] of variant rs5925. This study proved that the genotype E3/4 carriers had a less reduction of LDL when compared to E3/3 genotype.

Santos et al.[68] included 156 heterozygous familial hypercholesterolemia patients caused due to low-density lipoprotein receptor gene mutation from Brazil in their study and the result showed that there is a significant difference in the reduction of the low-density lipoprotein level below with the genotype AA when compared with GG and GA phenotype.

3.3. Serum Lipase:

3.3.1. Introduction:

Lipase [LPS], is an enzyme that produces alcohols and fatty acids by hydrolyzing the linkage of fat esters.[69] It is a single-chain glycoprotein having a molecular weight of 48,000 Dalton [Da] and with about 5.8 isoelectric point.[70] Even though there are three iosenzymes of lipase, L2 is considered to be clinically significant as they are more specific and sensitive.[69]

3.3.2. Synthesis:

Lipase is mainly synthesized from the pancreas and also from the stomach, pulmonary mucosa and intestine.[69,70] Most of the lipase in the serum which are active are derived from the pancreas.[70]

3.3.3. Properties of Lipase:

Lipase present in the serum are stable at room temperature for a period of 1 week with negligible loss of activity or stable for 3 weeks at 4˚C provided hemolysis has not occurred as hemoglobin inhibits the serum lipase activity.[69] Serum sample can also be stored in frozen state for several years without change in the activity of the lipase.[70]

3.3.4. Action of Lipase:

Lipase helps in formation of 2-monoglyceride intermediate and long-chain fatty acids by catalyzing the hydrolysis of dietary triglycerides partially.[69] Pancreatic lipase specifically acts on the 1 and 3 positions of the triglyceride molecule and the rate of the reaction is controlled by the protein co-enzyme colipase and bile salts.[69]

3.3.5. Normal levels:

The normal level of lipase is determined to be <38 U/L at 37 °C temperature.[69]

3.3.6. Factors affecting normal levels:

There are various factors that can influence the levels of lipase in the serum. This can lead to either increase in lipase level or decrease in lipase level. The various factors are enumerated below.

3.3.6.1. Hyperlipasemia:

The conditions that can lead to increase in serum lipase level include penetrating duodenal ulcer, perforation of peptic ulcer, intestinal obstruction, acute cholecystitis, acute pancreatitis, pancreatic duct calculus causing obstruction, pancreatic cancer, in patients with decreased glomerular filtration rate, Endoscopic Retrograde Cholangio Pancreatography [ERCP] and opioids.[69,70] Serum lipase may also be increased in conditions like cystic fibrosis, celiac disease, crohns disease.[71]

3.3.6.2. Hypolipasemia:

Factor that leads to decreased lipase level is destruction / damage to the pancreatic tissue which can lead to raised cholesterol and triglyceride levels, rise in blood pressure, difficulty in losing weight and varicose veins.[71]

3.3.7. Estimation of Lipase:

There are various methods available for measuring the activity of lipase and each follows different principles. The various techniques include

titrimetric, turbidimetric, spectrophotometric, fluorometric and immunologic techniques which use both the triglyceride and nontriglyceride substrates for the determination of lipase.[70]

The estimation of serum lipase done in this study is a recently used technique which follows the principle of direct reaction and have more specificity for pancreatic lipase.[70] Lipase catalyses the hydrolysis of 1,2-O-dilauryl-racglycero-3-glutaric acid-(4-methyl-resorufin)-ester which has two glycerol ether bonds and one ester bond to form 1,2-O-dialuryl-rac-glycerol + acid-(6'-methylresorufin)-ester. Then in the presence of water acid-(6'-methylresorufin)-ester is converted to glutaric acid and methylresorufin. The concentration is measured at 580 nm from the rate of the red dye formation.

3.3.8. Clinical Significance:

Measurement of serum lipase is useful in the diagnosis of acute pancreatitis [AP] as the clinical sensitivity is 94 %[71] and the clinical specificity is 80 to 100 % obtained from studies on varied population.[70] Serum level of LPS increases within 4-8 hours, reaches the peak by 24 hours and declines within a period of 7 to 14 days in case of AP.[70] It has been reported that the increase of serum lipase by 2 to 5 folds than the upper limit is diagnostic of acute pancreatitis and is more specific.[71]

Hence serum lipase estimation is recommended, over estimation of serum amylase [AMY] level as a diagnostic tool for acute pancreatitis and it

is not necessary to determine the levels of both serum lipase and amylase to confirm acute pancreatitis.[70]

3.4. Blood Glucose:

3.4.1. Introduction:

Glucose is the most essential substance from which energy is obtained for the normal functioning of the body tissue and continuous supply of energy to the brain cells is dependent on glucose level in the blood.[72] Derangement in the metabolism of glucose can lead to life threatening conditions. Blood glucose level ranging between 70 to 110 mg/dl in the fasting state is required for the normal functioning of tissues and cells.[72] A daily allowance of 160 grams [gms] is needed for the body to function normally, out of this 120 gms of glucose is required for the human brain to function.[73]

3.4.2. Sources of glucose:[73]

The main source of glucose is derived from the diet in the form of carbohydrates, fructose, galactose and mannose. Glucose is the main source of energy which is stored as glycogen and is then converted to glucose whenever there is a demand for glucose by the cells.

3.4.3. Synthesis:[73]

The glucose is mainly synthesized by three major metabolic pathways which includes gluconeogenesis, glycogenolysis and galactose metabolism.

3.4.3.1. Gluconeogenesis:[73]

It is defined as a synthesis of glucose from non-carbohydrate aminoacids like lactate and glycerol.

Gluconeogenesis mainly occurs in the cytosol and some occurs in the mitochondria. The process of gluconeogenesis takes place mainly in the liver and in kidney to some extent and is regulated depending upon the availability of glucagon and insulin.

Gluconeogenesis occurs during fasting and meets the basal requirement of glucose needed for the body. It also effectively eliminate the metabolites like lactate, glycerol and propionate that is produced in the tissue and accumulated in the blood.[73]

3.4.3.2. Glycogenolysis:[73]

It is a process of breakdown of glycogen to form glucose and is an irreversible reaction. Glycogen, a storage form of glucose is mainly stored in the liver [6-8%] and in the muscle [1-2%]. It is stored in the form of granules in the cytosol where the enzymes that are helpful in glycogen synthesis and breakdown are present. The main function of the glycogen

in the liver is to maintain the normal levels of blood glucose, especially between the meals. Glycogen gets stored in the liver during the surplus feeding period and gets utilized during the fasting state. Apart from this, the glycogen in the muscle helps in the formation of adenosine triphosphate [ATP] and serves as a fuel during the contraction of the muscle.

Even though fat is also a source of reserve energy for the body, glycogen is considered to be useful for day to day activity as it can be easily metabolized even in the absence of oxygen and utilized for the continuous supply of glucose.

3.4.3.3. Galactose metabolism:[73]

It is a process of formation of glucose from galactose along with the synthesis of lactose.

3.4.3.3.1. Sources of galactose:

The primary source of galactose is milk and milk products and is available as a disaccharide lactose in the diet.[73]

3.4.3.3.2. Metabolism:[73]

Galastose is formed in the cells by the degradation of glycoprotein and glycolipids. Lactase also known as β-galactosidase which is present in the intestinal mucosa hydrolyses lactose to galactose and then to glucose.

3.4.4. Glucose transport mechanism:[73]

For a normal functioning of the living cell, it is necessary that the formed glucose has to enter the cell from plasma. The concentration of glucose in the plasma is very high when compared to the glucose in the cells. The glucose that is present in the plasma cannot enter the cell by diffusion. There are two specific transport systems that help the transport of glucose from the plasma to the cells.

a. Insulin-independent transport system
b. Insulin-dependent transport system

3.4.4.a. Insulin-independent transport system:

This system of glucose transport is a carrier mediated uptake and is present in hepatocytes, erythrocytes and brain cells.[73]

3.4.4.b. Insulin-dependent transport system:

It is a glucose transport system which requires insulin for the transport of glucose in to the cells and occurs in the muscle and adipose tissue.[73]

3.4.5. Transporters of glucose:[72]

There are totally six transporters that are identified as transporters of glucose which includes GLUT-1, GLUT-2, GLUT-3, GLUT-4, GLUT-5 and GLUT-7. These transpoters are seen in the cell membrane and have

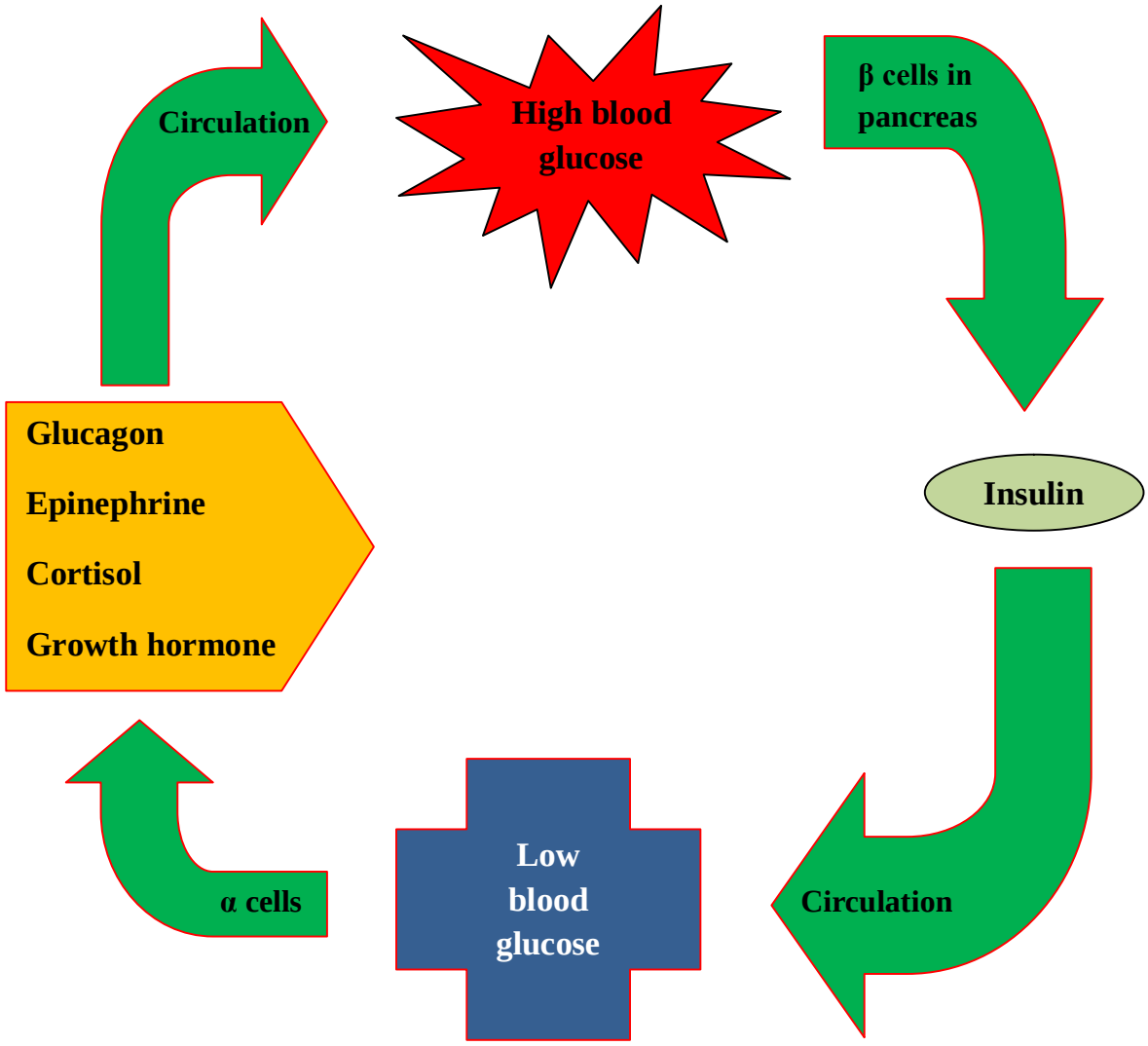
specificity towards each tissue. For example GLUT-1 is seen more in erythrocytes and GLUT-4 is seen in skeletal muscle and adipose tissue.

3.4.6. Regulation of blood glucose:

Blood glucose plays a major role in maintaining the normal functioning of the cell and there is a need for the plasma concentration of the glucose to be maintained within the normal range.[72]

The regulatory mechanism of blood glucose is schematically represented in Figure 4.

Figure 4. Diagrammatic representation of maintenance of blood glucose[74]



The blood glucose concentration is maintained in the normal range by various hormones that regulates the movement of glucose in and out of the circulation.[74] Insulin is the hormone that decreases the blood glucose level where as glucagon, epinephrine, cortisol and growth hormone, the counter-regulatory hormones increase the concentration of blood glucose.[74] Maintenance of blood glucose is diagrammatically represented in Figure 4.

3.4.7. Normal levels of blood glucose:[75]

Maintenance of blood glucose within the normal range is needed for the normal functioning of cells.

The normal levels of blood glucose and glycosylated hemoglobin [HbA1C] levels is shown in table 2.

Table 2. Normal blood glucose and glycosylated hemoglobin [HbA1C] levels[75]

	Normal	Pre diabetes	Diabetes Mellitus
Fasting Plasma Glucose	< 100mg/dl	100-125mg/dl	> 126mg/dl
Post Prandial Plasma Glucose	< 140mg/dl	140-199mg/dl	> 200mg/dl
HbA1C	< 5.6%	5.7-6.4%	≥ 6.5%

3.4.8. Factors affecting normal levels:

3.4.8.1. Hyperglycemia:

Diabetes mellitus [DM] is a metabolic disorder that present with hyperglycemia which is caused due to the reduction in the insulin secretion, glucose utilization and increased production of glucose.[75]

Kim et al.[76] in their study conducted on the disturbances of hormone and metabolism due to altered sleep has shown that disturbances or deprivation of sleep has higher association of developing obesity, diabetes, insulin resistance and changes in the

regulation of leptin and ghrelin.

Ulhoa et al.[77] have proved the association between metabolic syndrome and the work of people in day/night shift. It was also stated that the glycemic control among shift workers is poor when compared to non-shift workers and the risk associated with the development of type 2 DM also increases by two fold.

3.4.8.1.1. Causes of hyperglycemia:

The various causes of hyperglycemia includes type 1 DM, type 2 DM, maturity onset diabetes of the young, gestational DM, genetic defects, disease of the exocrine pancreas and Endocrinopathies, drugs, infections and genetic variations.[75]

The various causes of hyperglycemia is mentioned in table 3.

Table 3. Various causes of hyperglycemia[75]

Causes of hyperglycemia	
Type 1 DM	
Type 2 DM	
Gestational diabetes mellitus [GDM]	
Maturity onset diabetes of the young [MODY]	
Drugs	
➢ Glucocorticoids ➢ Nicotinic acid ➢ β-adrenergic agonists ➢ Diuretics [Thiazides] ➢ Adrenaline ➢ Diazoxide ➢ Antipsychotics	➢ mTOR inhibitors ➢ Hydantoins ➢ Asparginase ➢ α-interferon ➢ Protease inhibitors ➢ Calcineurin ➢ Vacor (rodenticide)
Infections	
➢ Coxsaxkievirus ➢ Cytomegalovirus ➢ Congenital rubella	
Diseases of the exocrine pancreas	
➢ Pancreatitis ➢ Cystic fibrosis ➢ Fibrocalculous pancreatopathy ➢ Hemochromatosis	➢ Neoplasia ➢ Mutations in carboxy ester lipase ➢ Surgical removal of Pancreas
Endocrinopathies	
➢ Hyperthyroidism ➢ Cushings syndrome ➢ Acromegaly ➢ Glucagonoma	➢ Pheochromocytoma ➢ Aldosteronoma ➢ Somatostatinoma
Genetic syndromes	
➢ Down's syndrome ➢ Porphyria ➢ Turner's syndrome	➢ Huntington's chorea ➢ Myotonic dystrophy ➢ Klinefelter's syndrome
Immune-mediated diabetes	
➢ Anti-insulin receptor antibodies	

3.4.8.1.2. Complications of hyperglycemia:[78]

Hyperglycemia can lead to acute complications like diabetic ketoacidosis and heperglycemic hyperosmolar state. Chronic hyperglycemia can lead to microvascular complications that includes retinopathy, nephropathy and neuropathy and macrovascular complications like coronary artery disease [CAD], peripheral vascular disease [PVD] and cerebrovascular disease [CVD].

Non vascular complications that can occur due to hyperglycemia includes infections, gastroparesis, diarrhea, uropathy, sexual dysfunction, hearing loss, cataract, glaucoma, periodontal disease, skin changes and cheiroarthropathy.

3.4.8.2. Hypoglycemia:

Hypoglycemia is a condition in which there will be a reduction in the blood glucose level below 70mg/dl which can lead to morbidity and in some cases it can be fatal.[79]

3.4.8.2.1. Types of hypoglycemia:[80]

Hypoglycemia is classified in to 4 types namely

a) Insulin-induced hypoglycemia
b) Postprandial hypoglycemia [Reactive hypoglycemia]
c) Fasting hypoglycemia
d) Alcohol-related hypoglycemia

3.4.8.2.1.a. Insulin-induced hypoglycemia:[80]

Diabetic patients who are treated with insulin for achievement of tight glycemic control are frequently affected. These patients, when conscious are managed by oral carbohydrate and unconscious patients are treated with subcutaneous or intramuscular glucagon or intravenous glucose.

3.4.8.2.1.b. Postprandial hypoglycemia:[80]

This is a common form of hypoglycemia caused due to excessive release of insulin followed by meal and usually the plasma glucose levels comes back to normal without any management. This can be avoided by having light meals frequently than having large three meals.

3.4.8.2.1.c. Fasting hypoglycemia:[80]

Fasting hypoglycemia occurs due to the decrease in the hepatic glycogenolysis or gluconeogenesis and is commonly seen in hepatocellular damage, adrenal insufficiency and in persons who have taken large volume of ethanol.

3.4.8.2.1.d. Alcohol related hypoglycemia:[80]

It mainly occurs due to ethanol mediated increase in Nicotinamide dinucleotide hydride [NADH] causing decreased synthesis of glucose by directing the intermediates like pyruvate and oxaloacetate in to the alternate pathways.

3.4.8.2.2. Causes of hypoglycemia:[81]

The various causes of hypoglycemia are classified depending upon the various age groups. The commonest causes of hypoglycemia in neonates includes small for gestational age or prematurity, toxemia of pregnancy, maternal diabetes mellitus, respiratory distress syndrome, cold stress and polycythemia. Various causes of hypoglycemia in infants includes ketotic hypoglycemia, congenital enzyme defects, glycogen storage disease, deficiency of gluconeogenic enzymes, galactosemia, hereditary fructose intolerance, leucine hypersensitivity, endogenous hyperinsulinism, Reye's syndrome and idiopathic causes. The various causes of hypoglycemia in adult who are under treatment with drugs, include insulin or insulin secretagogue, quinine, indomethacin, etc. Critical illness like hepatic, renal or cardiac failure, inanition, deficiency of glucagon, epinephrine and cortisol, sepsis and malaria can cause hypoglycemia. In adult who appears to be apparently normal, hypoglycemia can be due to endogenous hyperinsulinism, insulinoma, functional β-cell disorders and antibody to insulin secreted or to insulin receptors. Apart from these, hypoglycemia can be due to post gastric bypass, insulin secretagogues, insulin autoimmune disorder and non-insulinomal pancreatogenous etiology.

3.4.9. Estimation of blood glucose:

Estimation of glucose can be done by using serum plasma or whole blood. Nowadays, most of the laboratories use serum or plasma for

estimation of glucose.[81,82] The concentration of glucose in the plasma is 11% higher when compared to the concentration of glucose in the whole blood.[82]

The blood glucose can be estimated in the state of fasting for 8 to 10 hours, post prandial blood glucose after 2 hours of food and random blood glucose at any time of the day.[82]

Glucose estimation is most commonly done using hexokinase and glucose oxidase peroxidase methods. Other investigations for measurement of blood glucose includes modified glucose oxidase method and glucose dehydrogenase method.[81,82]

3.4.10. Stability of blood glucose:

The glucose level in the serum obtained from centrifuged clotted blood is decreased by 5 to 7 % in 1 hour at room temperature due to glycolysis. So fluoride is always added to the blood collected for estimation of glucose.[81]

3.4.11. Clinical Significance:[74]

The importance of monitoring the blood glucose levels in clinical practice was to diagnose and treat DM and its complications like diabetic keto acidosis [DKA], hyper osmolar non ketotic coma [HONK] and hypoglycemia. It also helps in monitoring the treatment of DM to restore the euglycemic state and to correct other metabolic disturbances associated with DM.

Estimation of blood glucose levels helps in knowing the present glycemic status in diabetic patients.

3.5. Pancreatitis:

3.5.1. Introduction:

Pancreas, a soft lobulated gland is located in the posterior abdominal wall behind the stomach consisting of two parts exocrine and endocrine and have less of connective tissue.[83] Pancreas is composed of the acini that secretes digestive juices and the islets of Langerhans that secrete insulin and glucagon.[84]

Pancreas is comprised of four major cells alpha [α] cells, beta [β] cells, delta [δ] cells and PP cells and two minor cells D1 cells and enterochromaffin cells.[83]

Alpha cells form 20 % of the total cells and are dense round granular cells which are responsible for the secretion of glucagon and have a diabetogenic property.[83] Beta cells are the cells that secrete insulin and have an anti-diabetogenic function.[83] They comprise of 68 % of the major cells.[83] Delta cells form 10% of the major cells and are responsible for the secretion of somatostatin that inhibits the release of insulin and glucagon.[83]

PP cells contribute to 2 % of the major cells, and are small and dark granules that secrete the hormone pancreatic polypeptide. This helps in the stimulation of gastrointestinal enzymes and inhibition of intestinal motility.[83]

D1 cells are responsible for the secretion of vasoactive intestinal polypeptide [VIP] which helps in inducing glycogenolysis and hyperglycemia. Vasoactive intestinal polypeptide also stimulates the secretion of gastrointestinal fluid which can lead to secretory diarrhoea.[83]

3.5.1.1. Physiology of pancreas:[84]

Pancreas is responsible for the secretion of two major hormones insulin and glucagon which are essential for the normal metabolism of glucose, lipid and protein. Apart from these hormones they also secrete digestive enzymes and other hormones like amylin, somatostatin, and pancreatic polypeptide.

3.5.2. Definition:

Acute pancreatitis is an acute inflammatory disorder of the pancreatic acinar cells[85,86] and usually presents with abdominal pain and elevation in the serum amylase and lipase levels.[85]

3.5.3. Prevalence:

The prevalence of AP ranges between 13 – 45 / 100000 person in a year and a population study revealed that there was a large increase in the AP incidence.[87]

The incidence of AP has grown up to 10 times during the past 20 years and the mortality due to AP ranges between 2 to 9 % and it depends upon the severity of the disease.[88]

3.5.4. Causes:

The various major causes that can lead to AP includes gall stones [30-60 %], alcohol [15-30 %], ERCP [5-20 %], hypertriglyceridemia [1.3-8.3 %] and drugs like azathioprine, 6-mercaptopurine, sulfonamides, tetracycline, anti HIV-drugs, estrogens, valproic acid and 5-aminosalycylic acid [2-5 %].[89]

The mechanism behind the drug induced AP is either by hypersensitivity reaction or due to the toxic metabolite generation.[90]

Some of the other causes of AP includes blunt trauma to the abdomen and abdominal and non abdominal surgeries. The uncommon causes of AP includes vasculitis, connective tissue disorders, thrombocytopenic purpura [TTP], hypercalcemia, periampullary diverticulum, pancreas divisium, cancer of the pancreas, hereditary pancreatitis, cystic fibrosis of pancreas, renal failure, viral infections, parasitic infection and type1 and 2 auto immune disorder.[89]

Apart from the above mentioned drugs that can cause AP, there are case reports on statin induced AP.

Anagnostopoulos et al.[9] reported a case of AP in a 56 year old patient from Greece who was treated with pravastatin 20mg for a period of 6 months where other causes of AP were ruled out. The patient again reported with AP after 5 months duration which recurred after 3 days of initiating pravastatin self medication.

Chintanaboina et al.[10] reported a 67 year old female on rosuvastatin and clonidine who presented with abdominal pain and vomiting for 7 days and investigation confirmed AP. The patient was treated with conservative management and medications were stopped during the stay in the hospital. The patient responded and was discharged with an advice to continue her routine medications but she again presented with AP after 2 months. After thorough workup the possible cause of AP was suspected to be rosuvastatin and the drug was withdrawn and she was completely asymptomatic during the 18 months follow-up.

Singh et al.[11] reported about a case of AP in a 77 year old female who was on rosuvastatin which subsided on stopping the drug. Detailed treatment history revealed that she suffered a similar episode a year back which was precipitated by atorvastatin.

Antonopoulos et al.[12] reported about a 58 year old Caucasian male on treatment with acetyl-salicylate for 6 years and simvastatin for the past 2 months who presented with epigastric pain and vomiting and investigations revealed AP.

Tsigrelis et al.[13] in their case report stated about a 50 year old female who developed AP following 3 days of treatment with pravastatin where all the other possible causes of AP were ruled out.

Etienne et al.[14] reported a case of a 58 year old male patient who presented with abdominal pain and vomiting who was under valproic acid,

omeprazole and simvastatin. Investigation revealed that serum amylase and lipase to be elevated which was suggestive of AP. Simvastatin was suspected to be the cause of AP and was stopped, which resolved the symptoms and his lipase dropped to the normal level.

Suwansiripat et al.[91] reported about an 84 year old Thai man who was a known case of hypertension, CAD and benign prostatic hypertrophy. He was under treatment with simvastatin, aspirin, amlodipine, trimetazidine hydrochloride and afluzosin. He was admitted in the hospital with acute pain over the epigastric region which was radiating to the middle of back. The pain started after taking the dinner and investigation carried out revealed that pancreatic amylase and liver function test were higher. Multi detector computerized tomography [MDCT] findings were suggestive of acute non-necrotizing pancreatitis.

Prajapati et al.[92] in their case report mentioned about a 58 year old hyperlipidemic male who was on atorvastatin. The patient developed acute abdominal pain and vomiting. Clinical examination of the abdomen revealed epigastric tenderness and investigations showed elevated lipase levels and CT was suggestive of acute pancreatitis. The patient recovered following conservative management and after withholding atorvastatin. The causality assessment of ADR was probable according to Naranjo and WHO-UMC scale.

3.5.5. Clinical Features:

3.5.5.1. Symptoms:[93,94]

Patients who have an episode of AP usually presents with severe abdominal pain usually confined to the epigastric region, but can also present all around the abdomen or lower chest. The pain is “knifing” or “boring through” in nature which radiates to the back and the pain may be relieved by leaning forward.

The patient will have vomiting and retching even after the stomach is empty, but there will be no relief for the pain.

Fever is another symptom associated with AP.

3.5.5.2. Signs:[93]

The signs that can be elicited on examination of the patients include tachycardia, tachypnea, hypotension, hyperthermia, distended abdomen due to intraperitoneal fluid, voluntary and involuntary guarding of the abdomen, reduced or absent bowel sounds, and left sided pleural effusion.

3.5.6. Investigations:

3.5.6.1. Laboratory investigations:

Laboratory investigations that are helpful in the diagnosis of AP include serum amylase, serum lipase, phospholipase A_2 [PLA_2],

trypsin/trypsinogen, carboxylester lipase, carboxypeptidase A, colipase, elastase, ribonuclease, pancreatitis associated protein [PAP], methemalbumin, white blood cell [WBC] count, blood glucose level, serum glucagon levels, serum aspartate transaminase [AST], alanine aminotransferase [ALT], alkaline phosphatase, bilirubin, serum triglyceride level, C-reactive protein, interleukin-6 [IL-6], polymorphonuclear leukocyte elastase, urinary trypsinogen activation peptide and procalcitonin.[95]

Apart for these investigations complete blood count, blood urea nitrogen [BUN], serum calcium levels, arterial blood gas [ABG] analysis and urinalysis are carried out.[96]

Serum amylase and lipase are considered to be the markers that is used to diagnose AP[97] and they tend to increase by 3 fold than that of the normal value.[95] Serum lipase is more specific for AP when compared to serum amylase, as serum amylase is found to be increased in other conditions like salivary gland dysfunction, tumors of salivary gland, macroamylasemia, gynaecological diseases, intestinal perforation, intestinal infarction, chronic alcoholism, post operative state and renal failure.[95,98]

There will be an increase in the WBC count, blood glucose level, serum glucagon, AST, ALT, alkaline phosphatase, serum bilirubin levels and serum triglycerides.[95,97]

PAP is a heat shock protein which is not seen in persons with normal pancreatic function, whereas it is remarkably raised in AP and the accuracy of identifying the PAP is similar to that of serum amylase.[95]

3.5.6.2. Radiological investigations:

3.5.6.2.1. Radiography:[95]

Plain X-ray of the abdomen suggests both the etiology and severity of acute pancreatitis. It is helpful in diagnosing the conditions like gall stones, pancreatic stones, pancreatic calcification, ascitis and gas in the retroperitoneum.

X-ray of the chest in patients with acute pancreatitis shows pleural effusion, pulmonary infiltrates, elevated hemidiaphragm, basal or plate-like atelectasis which was due to reduction in the respiratory excursion. The abnormality in the roentgenogram of chest is seen in about 30% patients with acute pancreatitis.

3.5.6.2.2. Ultrasonography [USG] of abdomen:[95]

It is useful during the initial 24 hours of onset of symptoms in identifying the gall stones, dilation of bile duct, ascites and diagnosing the abnormalities in the pancreas.

The pancreas may be enlarged and hypoechoic.

3.5.6.2.3. Computerized Tomography [CT]:[95]

CT is considered to be the important radiological test in diagnosing AP and its complications related to abdomen. The three major indications to proceed for a CT is to exclude other abdominal conditions, staging the severity of AP and to identify the other abdominal complications of AP.

3.5.6.2.3.1. Grading system:[95]

There are two main systems which help in grading the severity of the disease by CT i.e. grading system of Balthazar and CT severity index [CTSI].

3.5.6.2.4. Magnetic Resonance Imaging [MRI]:[95]

MRI is also helpful in obtaining the details about the severity of AP as CT does. MRI is helpful in identifying the necrosis of the pancreas and fluid collections in the abdomen. It is much better than CT and is equally good as ERCP and endoscopic USG.

3.5.7. Predictors of severity:[95]

CT and MRI plays a major role in predicting and grading the severity of pancreatitis and is useful in the early stage of the disease in order to prevent the complications and to improve therapy.

There are various scoring systems which helps in grading the severity of pancreatitis which includes Ranson's score, acute physiology and chronic health evaluation II [APACHE II] scores, bedside index of severity in acute pancreatitis [BISAP], blood urea nitrogen [BUN], organ failure and peritoneal lavage to rule out infection.

3.5.8. Treatment:

The main role of treatment in AP is the alleviation of pain, correction of fluid volume and metabolic abnormalities, inhibition of enzymes, secretions and inflammation and supplementation of nutrition.[99,100]

3.5.8.1. Management of pain:

The most important sign of AP is the pain and the management of pain should be the foremost aim clinically. Non steroidal anti-inflammatory drugs [NSAIDs] are useful in the management of mild pain, where as weak opioids are helpful in patients having moderate to severe pain and in patients where NSAIDs did not relieve the pain. Patient who did not respond to weak opioids can be put on strong opioids.[99]

Metamizole, an NSAID of choice given at a dose of 2 gram/8hour [h] intravenously [IV], Buprenorphine, an opioid of choice given at a dose of 0.3 g/4 h IV are the first choice of analgesics.[99] Meperidine was traditionally used as it does not alter the function of spincter of Oddi significantly and will not cause pancreatitis.[100]

3.5.8.2. Fluid management:

Resuscitation with fluid plays a vital role in the management AP during the early stage and helps in improving the clinical outcome.[99] Intravenous fluid and electrolytes replacement plays a major role and dextran helps in expanding the intravascular volume and improves the blood flow to the pancreas.[100]

The main goal is to maintain the blood volume in the circulation in order to maintain the heart rate, blood pressure, central venous pressure and renal function by maintaining the urine output of >0.5 ml/h/kilogram [kg] body weight which is essential in Management.[99]

Zhao et al.[101] conducted a randomized controlled trial involving 120 patients with severe acute pancreatitis [SAP] who were randomized in to three groups namely normal saline [NS] [NS group], NS and hydroxyethyl starch [HES] [SH group] and NS, HES and glutamine [SHG group] and resuscitation was done. The author finally concluded that the combination of SHG in management was found to be most efficient among these in reducing the inflammation and retaining the intestinal barrier in SAP.

3.5.8.3. Management of metabolic abnormalities:[100]

Decrease in the levels of serum calcium is noted in AP and affects the prognosis of the disease if the level is < 7.5 mg/dl and hence administration of calcium at slow rate is considered.

AP itself can lead to hyperlipidemia and vice versa, so therapy should not be started for hyperlipidemia during an attack of AP. Re-evaluation of hyperlipidemia after the recovery from an episode of AP must be done and then therapy can be considered.

3.5.8.4. Inhibition of enzymes, secretion and inflammation:[100]

Gabexate mesilate, a protease inhibitor was the first agent tried in humans to inhibit the pancreatic enzymes. Studies conducted with this drug have shown benefit which was not significant statistically. Gabexate showed to reduce the complications of AP by 30 %.

Somatostatin and octreotide, the inhibitors of pancreatic secretion underwent various clinical trials and a meta-analysis showed that there was a reduction in the mortality which was statistically significant with somatostatin but not with octreotide which was not statistically significant.

3.5.8.5. Nutrition:

Nutrition to the patient with AP can be administered either by enteral route or parenteral route. Nasojejunal feeding is recommended during the episode of AP which helps in early initiation of oral intake and also improves the outcome of severe disease.[100]

Enteral nutrition is considered to be the ideal route for administering nutrition as it provides rest to the pancreas. The wide range

of advantages of enteral nutrition over parenteral nutrition includes good glycemic control, decrease in the complications due to infection, reduction in the need for surgery and decrease in the mortality.[102]

The ideal site of enteral administration is to place the tube by nasojejunal and nasogastric route.[102] Even though traditional method is to start oral feed with low-fat diet when the patient is capable of taking oral feeds, evidence states that low-fat diet will not affect the recovery of the patient.[100]

The enteral formulations that are available for administration of nutrition in AP are classified broadly into elemental formulation which includes amino acids, oligopeptides, maltodexrtrins and medium- and long-chain triglycerides.[102]

Polymeric formulation includes nonhydrolyzed proteins, maltodextrins, oligofructosaccharides and long-chain triglycerides.[102]

Immune enhancing formulation includes glutamine, arginine, omega-3 fatty acids, probiotics and fibre-enriched formulations which are hypothesized to modulate the immune system.[102]

3.5.9. Complications:[89]

Complications due to AP are basically classified into local and systemic complications.

Local complications of AP are pancreatic necrosis that may be sterile or infected, walled-off necrosis, pancreatic pseudocyst, collection of pancreatic fluid, pancreatic duct disruption, pancreatic ascites, involvement of contiguous organs, thrombosis of portal and splenic veins, pancreatic enteric fistula, bowel infarction and obstructive jaundice.

Systemic complications due to AP include pleural effusion, atelectasis, mediastinal fluid, pneumonitis, acute respiratory distress syndrome and hypoxemia. Cardiovascular complications include pericardial effusion, hypotension, hypovolemia and non specific ST-T changes in electrocardiogram suggestive of myocardial infarction. Hematological complications include disseminated intravascular coagulation, GI complications include peptic ulcer disease, gastritis due to erosion, portal and spleenic vein thrombosis and portal variceal hemorrhage. Renal complications include oliguria, azotemia, renal artery and vein thrombosis and acute tubular necrosis.

3.5.10. Prognosis:

Imaging techniques play a major role in the prediction of prognosis of AP.[103]

The mortality due to severe AP is 15% to 30% and mild pancreatitis is 0% to 1% which is mainly due to organ failure.[104] The chance of secondary infection that occurs 3 to 4 weeks after the occurrence of

necrotizing pancreatitis is around 30% and its mortality goes up to 100% if left untreated.[104]

Around 50% of deaths due to AP occur during the first seven days of an attack. The proportion of the survivors during the first week of the disease takes a sequence of clinically worst prognosis of pancreatic or peripancreatic necrosis. This can lead to secondary infection causing sepsis and multi organ dysfunction syndrome and finally leads to death of the remaining survivors.[105]

The prognosis of mild case of AP is good as the recovery is complete in almost all the cases.[105]

Cetinkaya et al.[106] in their study conducted on 102 patients with AP identified platelet ratio to be a worthy and newer test to predict the prognosis of AP.

Fisic et al.[107] conducted a study to evaluate a diagnostic test which is valuable to assess the severity and course of AP and confirmed that interleukin [IL]-6, IL-8, IL-10 and soluble receptor for tumor necrosis factor [sTNFr] on the day 1. CRP and elastase on third day is also a valuable predictor of AP.

Nieminen A et al.[108] who conducted a study on 163 patients with AP have proven that evaluation of the levels IL-6 and hepatocyte growth factor [HGF] at the time of admission can predict the prognosis of severe AP. These markers are also similar to that of CRP, creatinine and calcium

levels. Estimating the levels of IL-8, HGF and granulocyte colony stimulating factor [G-CSF] at the time of admission can also be used as predictors of severe AP without signs of organ dysfunction.

4. Methodology:

A prospective study was conducted on 71 patients in the Department of Pharmacology in collaboration with the Department of General Medicine, Sree Mookambika Institute of Medical Sciences [SMIMS], Kulasekharam, Kanyakumari District, Tamil Nadu for a period of one year from June 2014. The study protocol was approved by Institutional Human Ethics Committee [IHEC] [Ref No. SMIMS/IHEC/2013/C/13] and was submitted online to the Clinical Trial Registry - India [CTRI] [http://ctri.nic.in/clinicaltrials/login.php] and registered [**CTRI/2014/07/004714** dated 04/07/2014].

4.1. Inclusion and Exclusion Criteria:

The criteria for inclusion of patients in the study were hyperlipidemic patients with total cholesterol [200 to 500 mg/dl], triglycerides [150 to 600 mg/dl], low density lipoprotein [100 to 400 mg/dl], high density lipoprotein [<55 mg/dl], very low density lipoprotein [10 to 33 mg/dl] of both sex between the age of 20-60 years. Alcoholic patients with family history of pancreatitis, pancreatic cancer, diabetes, hypertension, who had undergone Endoscopic Retrograde Cholangiopancreatography [ERCP], renal failure, drug intake pregnant and lactating women were excluded from the study.

4.2. Parameters:

The parameters that were observed during the study were serum lipase in U/L and random blood sugar [RBS] in mg/dl.

4.3. Procedure:

The study was done in the General Medicine outpatient clinic of SMIMS hospital and 71 patients who were willing to participate in the study after satisfying the inclusion and exclusion criteria were included in the study. The patients were explained about the study and the procedure in detail and written informed consent was obtained from them before enrolling in the study.

After the consenting process, each patient was taken to the central laboratory, and 3 ml of venous blood was collected by using 5 ml sterile disposable syringe by the normal phlebotomy procedure. The collected blood was then transferred to the test tube containing clot activator and was allowed to stay for 20 minutes [mins] or till the blood clotted.

Image 1. Blood sample collected in clot activator tube

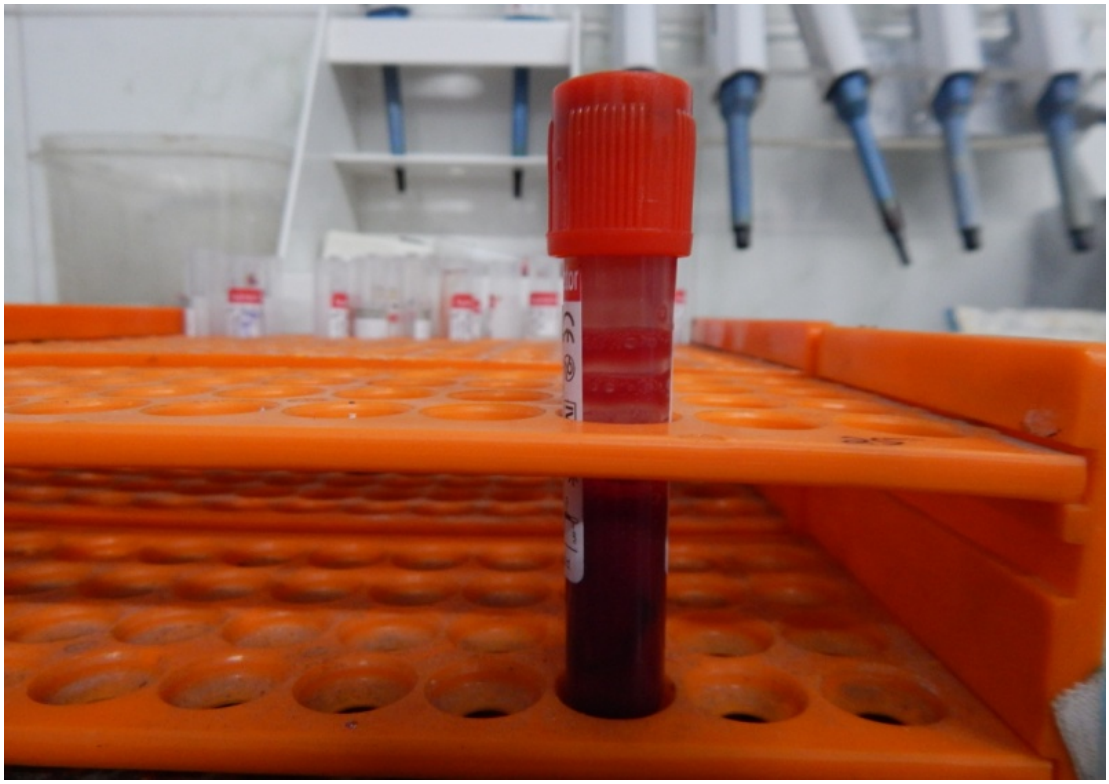
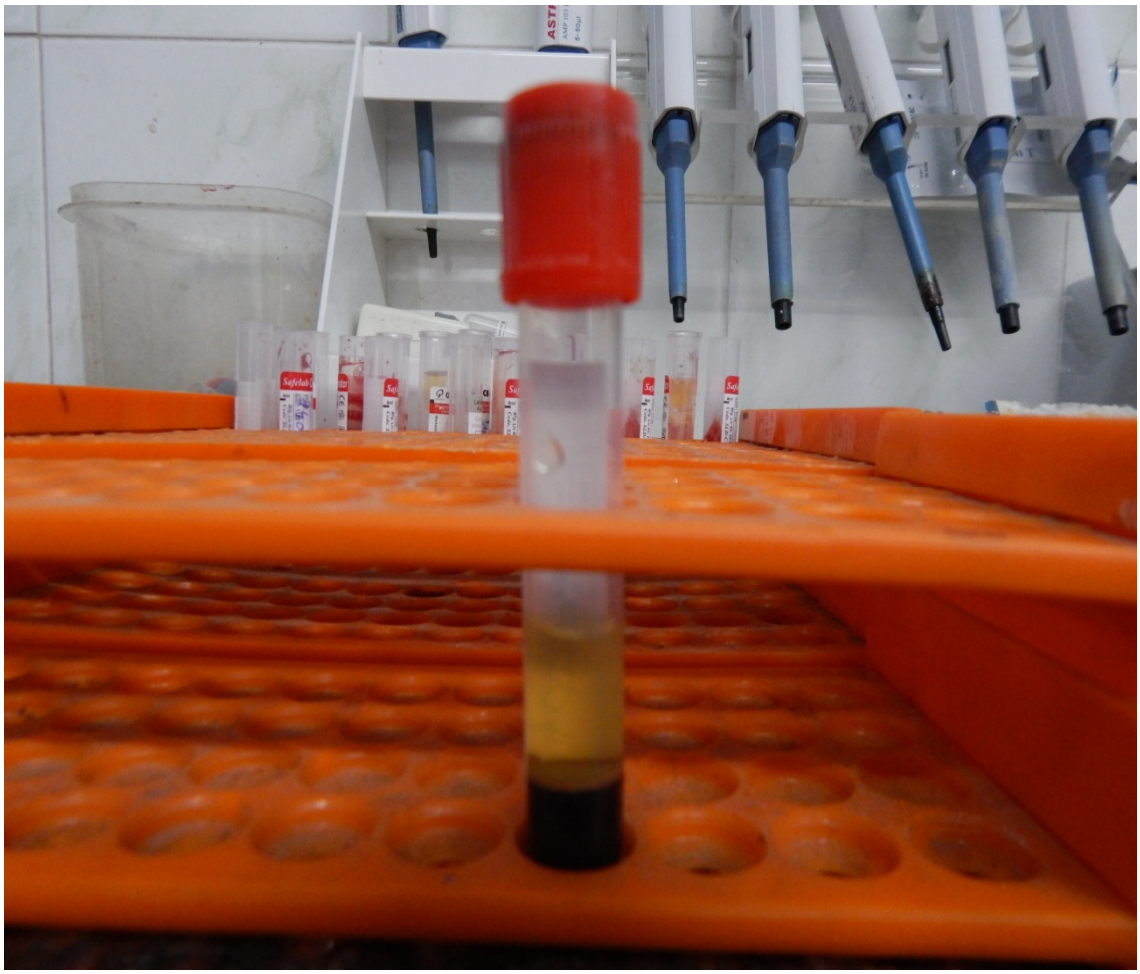
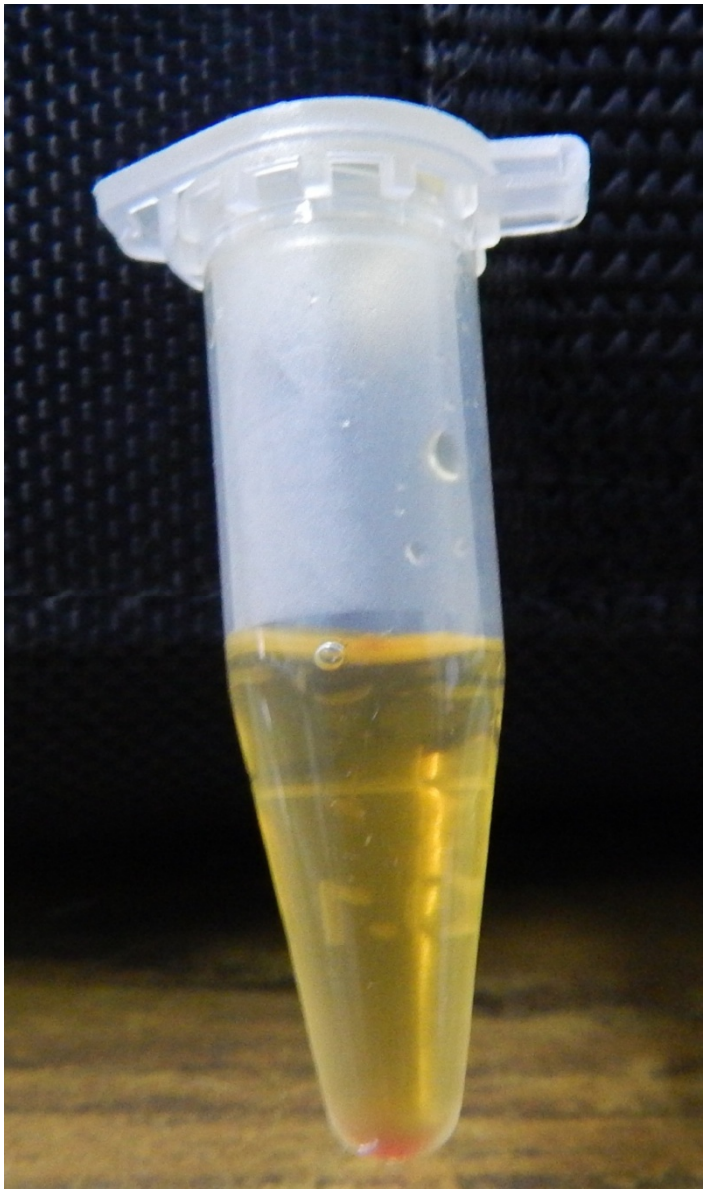


Image 2. Clotted blood in clot activator tube after 20 minutes



The clotted blood was then centrifuged at the speed of 2500 rotations per minute [rpm] for 5 minutes [mins] using Remi R – 8C centrifuge manufactured by Remi Elektrotechnik Ltd. [Instrument Division], Vasai – 401 208, India, for the serum to get separated from the blood. The serum as a supernatant solution was collected using a 1000 micro liter [µl] pipette and transferred into 1.5 ml eppendorf safe-lock tube and used for analysis of serum lipase and blood glucose levels.

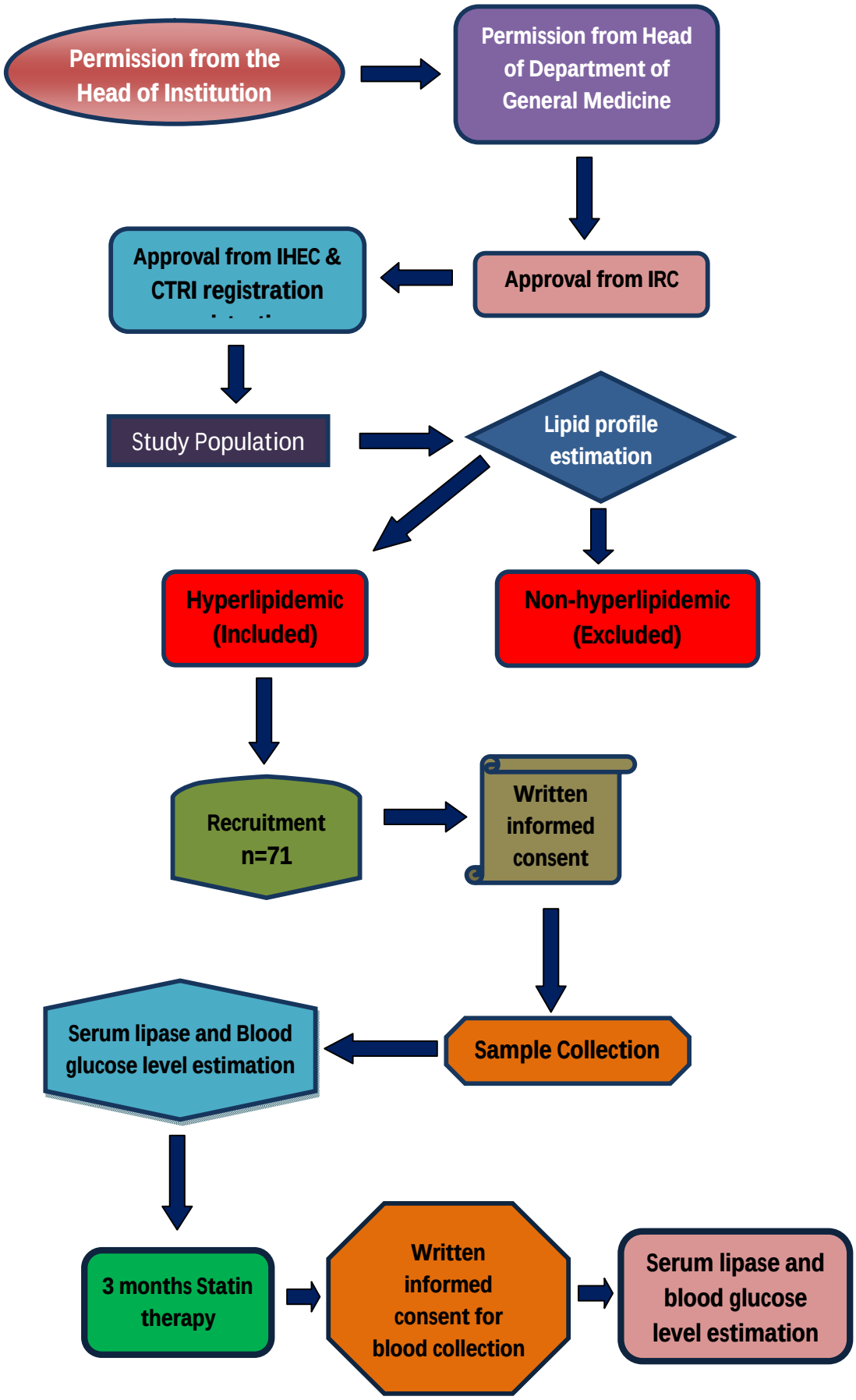
Image 3. Separated serum sample in eppendorf safe-lock tube



Patients were initiated on statin therapy as advised by the physician for a period of three months. They were requested to come for review after 3 months. At the time of review, informed consent was again obtained for collection and 3 ml venous blood was collected from each patient for estimation of serum lipase and blood glucose levels.

The study procedure is schematically presented in Figure 5.

Figure 5. Schematic diagram of the study procedure.



4.4. Estimation of Serum Lipase:

Estimation of serum lipase was done by kinetic method as described by Lipase color reagent manufactured by Biosystems S.A., Costa Brava 30, Barcelona [Spain], supplied by Star Diagnostic Supplies and Surgicals, Nagercoil and analyzed by using fully automated biochemistry analyser [BECKMAN COULTER Chemistry analyzer AU 480 manufactured by Beckman Coulter Mishima K.K. Beckman Coulter, Inc. 250 S Kraemer Blud, Brea, CA 92821, USA].

4.5. Estimation of Blood Glucose:

The estimation of blood glucose was done by using Glucose Monoreagent LR liquid reagent manufactured by GESAN production s.r.l. Italy, supplied by Star Diagnostic Supplies and Surgicals, Nagercoil and the analyzer used was Gesan Chem 200 Clinical chemistry autonalyzer [manufactured by Gesan, 71, Fiera dell'Eremita Street – 91021 Campobello di Mazara – Italy]. The estimation follows Colorimetric enzymatic glucose oxidase peroxidase [GOD-POD] method described by the manufacturer.

4.6. Statistical analysis:

The demographic data of patients age, height [Ht], weight [Wt], Body Mass Index [BMI], serum lipase in U/L and blood glucose in mg/dl were expressed in mean±standard deviation [mean±SD]. The distribution of sex was expressed in number [No.] and percentage [%].The data were entered into the Microsoft Office Excel 2007 for windows 7.

- GraphPad Instat version 3.06, 32 bit for Windows [GraphPad Software, San Diego, California USA] was used to analyze the data
- For data with normal (Gaussian) distribution parametric test, paired 't' test was used to find out the statistical significance between the data before and after 3 months of statin therapy
- For data with non-normal (Non-Gaussian) distribution non-parametric test, Wilcoxon signed rank test was used to find out the statistical significance between the data before and after 3 months of statin therapy
- $P < 0.05$ was considered as statistically significant
- The results in table and bar diagrams were presented as Mean ± SD

5. Results:

5.1. Study subjects:

Total number of participants recruited in this study was 71 after considering inclusion and exclusion criteria. The baseline parameters of the eligible participants were recorded before starting the statin therapy and the same were repeated after 3 months of statin therapy.

The baseline characteristics of the study subjects are depicted in Table 4.

5.2. Assessment of changes in serum lipase levels in all participants of the study:

In this study, the serum lipase levels were significantly increased after 3 months of statin therapy when compared to the baseline values [P<0.0001]. The changes in serum lipase levels before and after 3 months of statin therapy has been shown in figure 6.

5.3. Assessment of changes in blood glucose levels in all participants of the study:

The changes in blood glucose levels before and after 3 months of statin therapy has been illustrated in figure 7. There was statistical significant rise in blood glucose levels after 3 months of statin therapy when compared to the baseline values [P<0.0005].

5.4. Assessment of changes in serum lipase levels in male participants:

In this study, the serum lipase was increased after 3 months of statin therapy when compared to the baseline values. However this was not found statistically significant when compared to the baseline values [P>0.05]. The changes in serum lipase levels before and after 3 months of statin therapy in male participants has been delineated in figure 8.

5.5. Assessment of changes in blood glucose levels in male participants:

The study showed increase in blood glucose levels after 3 months of statin therapy when compared to the baseline values. However this was not found statistically significant when compared to the baseline values [P>0.05]. The changes in blood glucose levels before and after 3 months of statin therapy in male participants has been represented in figure 9.

5.6. Assessment of changes in serum lipase levels in female participants:

The changes in serum lipase levels before and after 3 months of statin therapy in female participants has been depicted in figure 10. The study showed statistically significant increase in serum lipase levels after 3 months of statin therapy when compared to the baseline values [P<0.0001].

5.7. Assessment of changes in blood glucose levels in female participants:

The changes in blood glucose levels before and after 3 months of statin therapy in female participants has been illustrated in figure 11. The study showed statistically significant increase in blood glucose levels after 3 months of statin therapy when compared to the baseline values [P<0.004].

Table 4: Baseline characteristics of the study subjects

	Mean±SD
Age (in Years)	41.59±8.71
Male (%)	35(49.3)*
Female (%)	36(50.7)*
BMI	26.56±2.41
Serum lipase (Units/L)	25.54±4.96
Blood glucose (mg/dl)	104.56±8.38
Total cholesterol (mg/dl)	255.20±34.01
Triglycerides (mg/dl)	192.73±44.78
LDL (mg/dl)	140.11±32.82
HDL (mg/dl)	36.46±5.46
VLDL (mg/dl)	38.80±8.85

Data are represented as **Mean ± SD**

*****Values are expressed in number (percentage) [%]

n = 71

BMI was calculated by using the following formula:

BMI= Weight (Kg)/Height in meter square

BMI: Body Mass Index

L: Litres

mg: milligrams

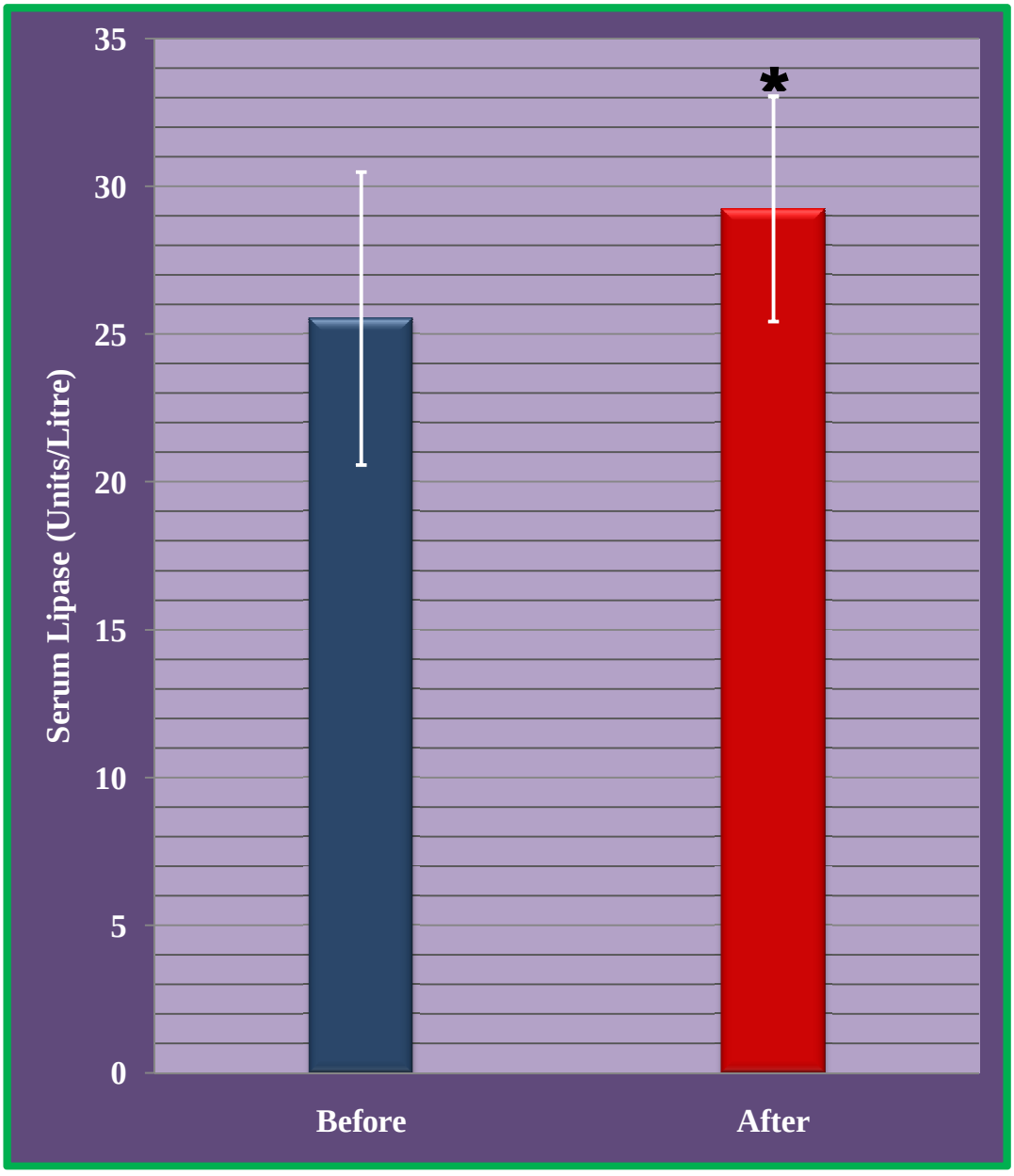
dl: decilitres

LDL: Low Density Lipoproteins

HDL: High Density Lipoproteins

VLDL: Very Low Density Lipoproteins

Figure 6. Bar diagram depicting the serum lipase [Units/Litre] levels before and after 3 months of statins therapy in hypercholesteremic patients



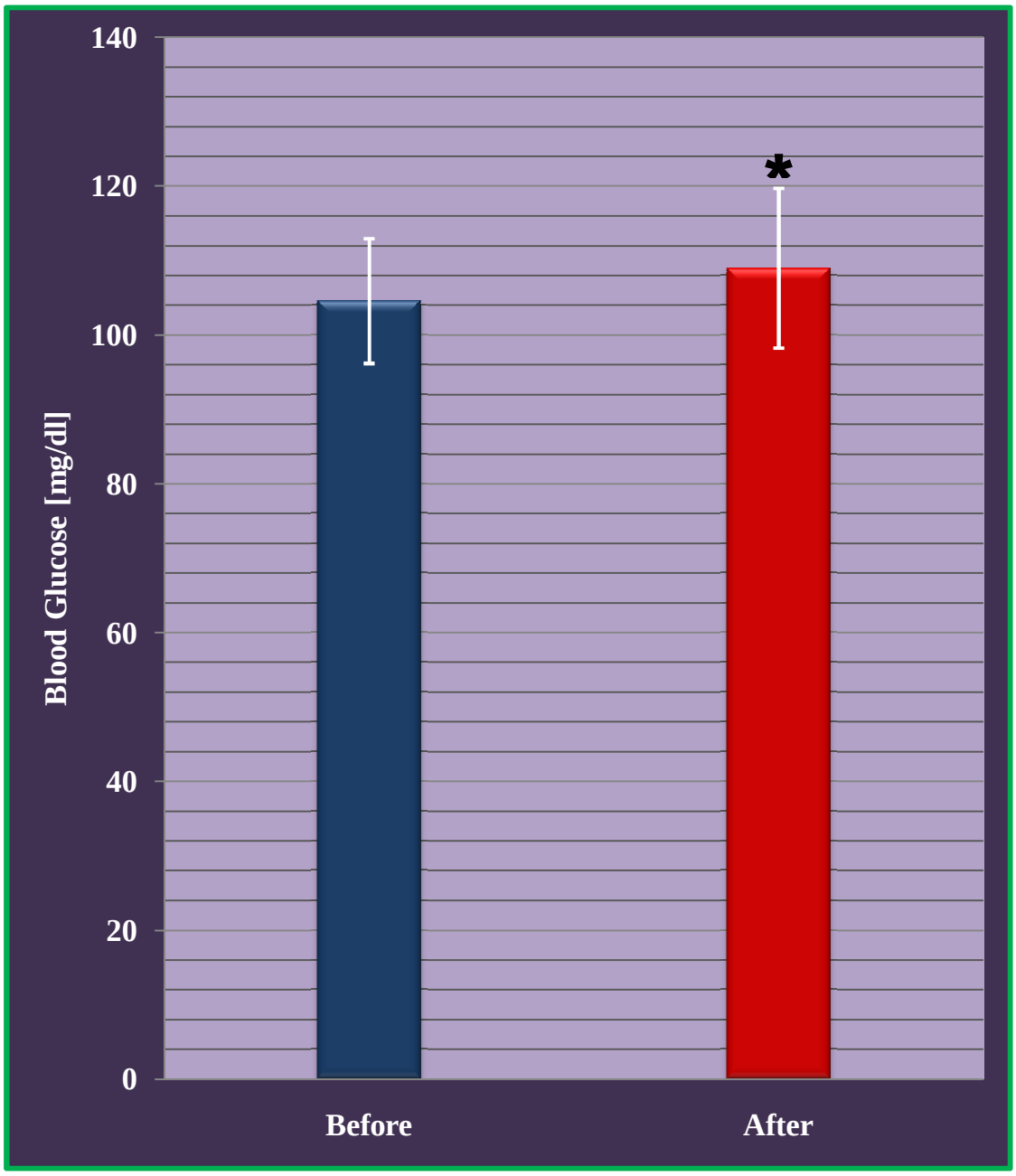
Data are represented as **Mean ± SD**

n = 71

***P** < 0.0001 when compared to the values before the start of statins

Data are analysed by using non-parametric Wilcoxon signed rank test

Figure 7. Bar diagram depicting the blood glucose [mg/dl] levels before and after 3 months of statins therapy in hypercholesteremic patients



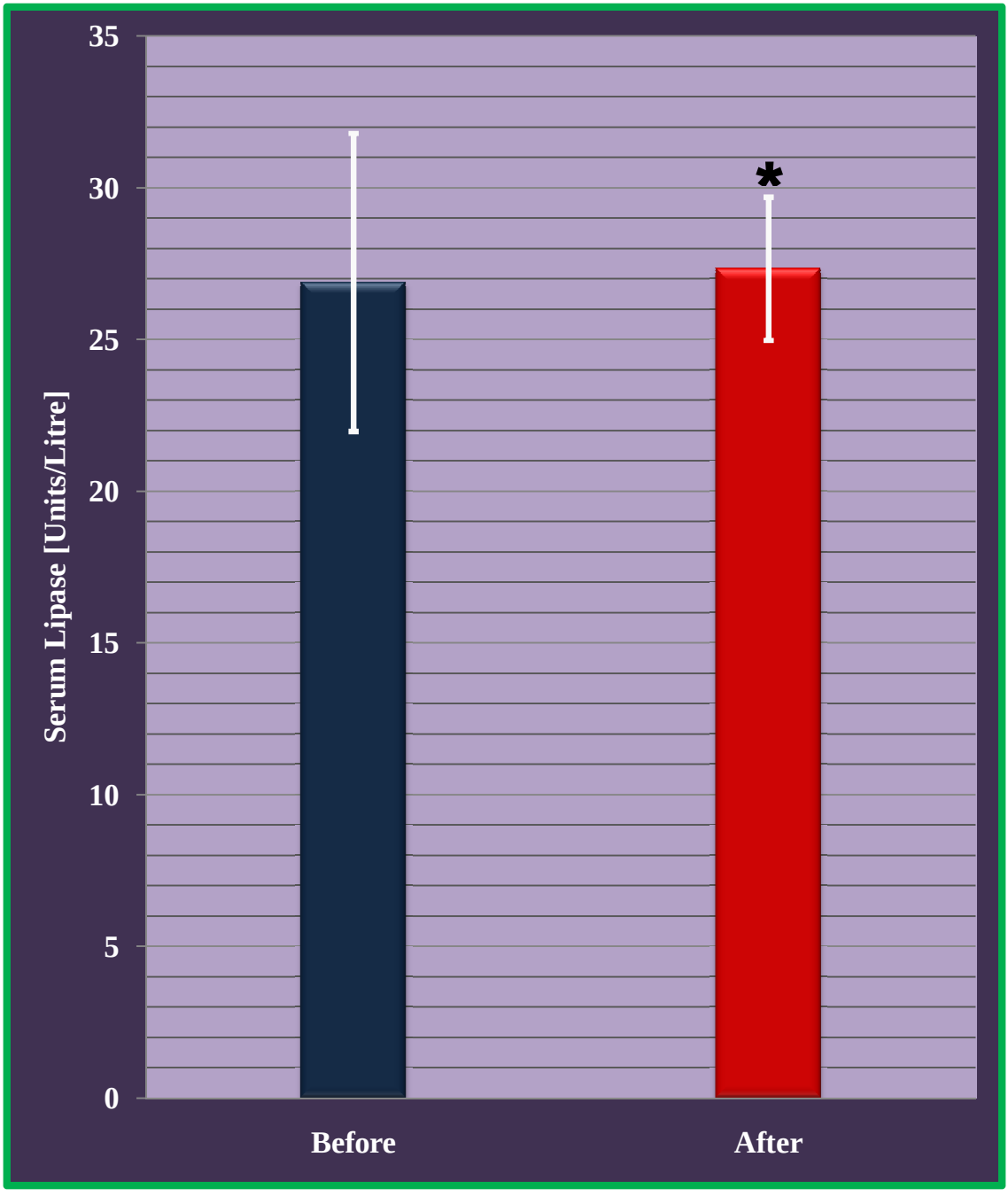
Data are represented as **Mean ± SD**

n = 71

***P** < 0.0005 when compared to the values before the start of statins

Data are analysed by using non-parametric Wilcoxon signed rank test

Figure 8. Bar diagram depicting the serum lipase [Units/Litre] levels before and after 3 months of statins therapy in male hypercholesteremic patients



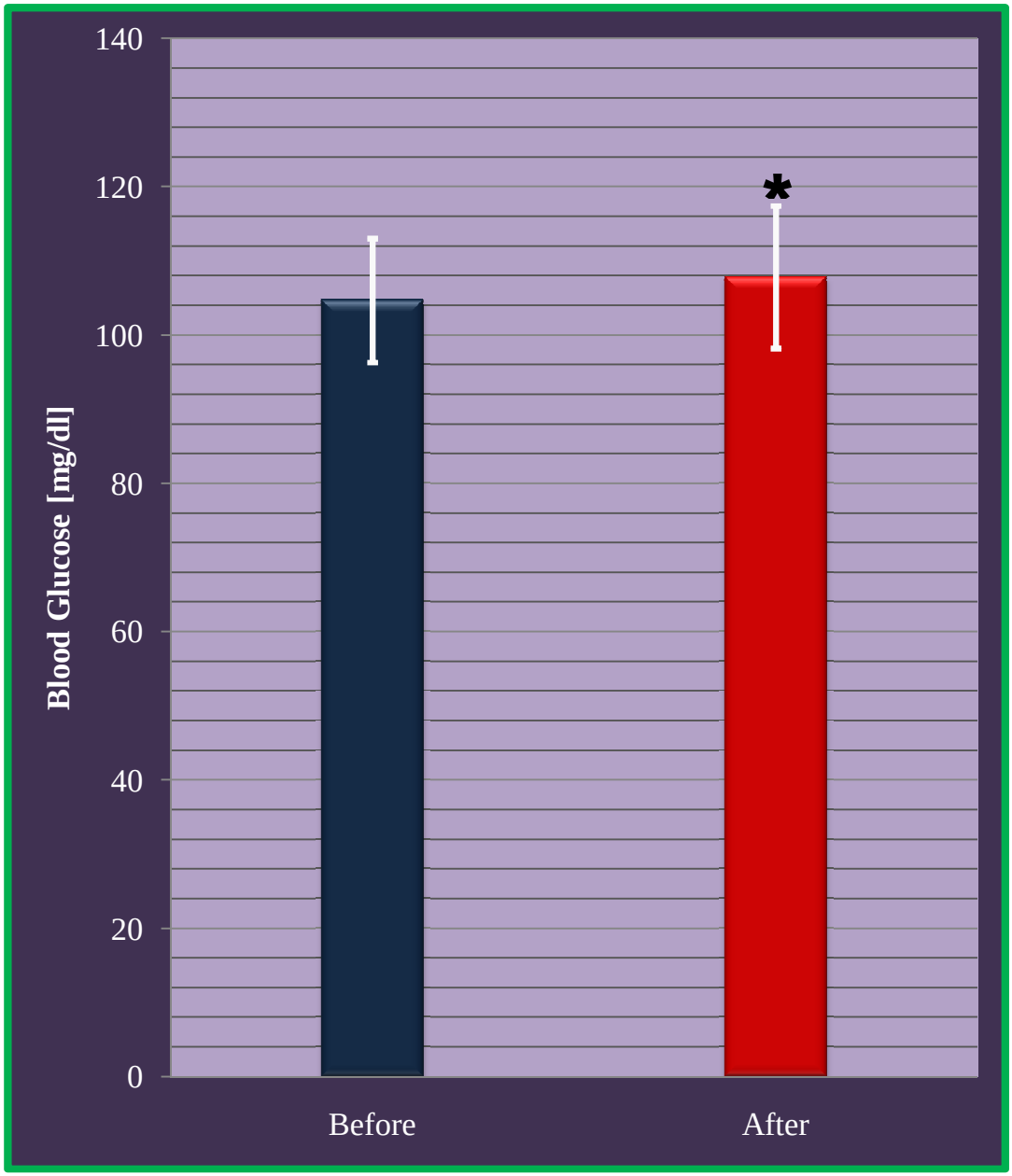
Data are represented as **Mean ± SD**

n = 35 [Male hypercholesteremic patients]

***P** > 0.05 when compared to the values before the start of statins

Data are analyzed by using paired 't' test

Figure 9. Bar diagram depicting the blood glucose [mg/dl] levels before and after 3 months of statins therapy in male hypercholesteremic patients



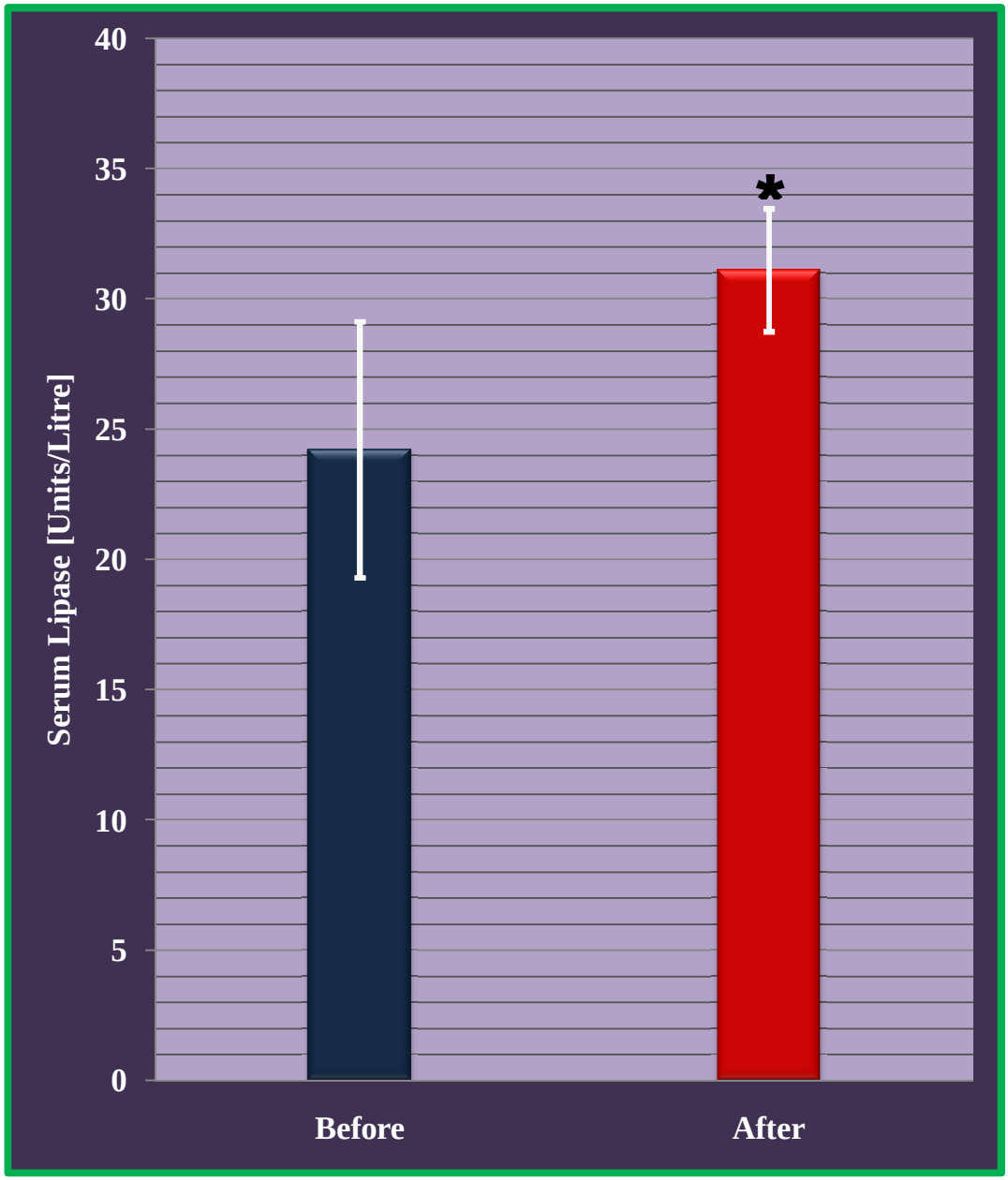
Data are represented as **Mean ± SD**

n = 35 [Male hypercholesteremic patients]

***P** > 0.05 when compared to the values before the start of statins therapy

Data are analyzed by using paired 't' test

Figure 10. Bar diagram depicting the serum lipase [Units/Litre] levels before and after 3 months of statins therapy in female hypercholesteremic patients



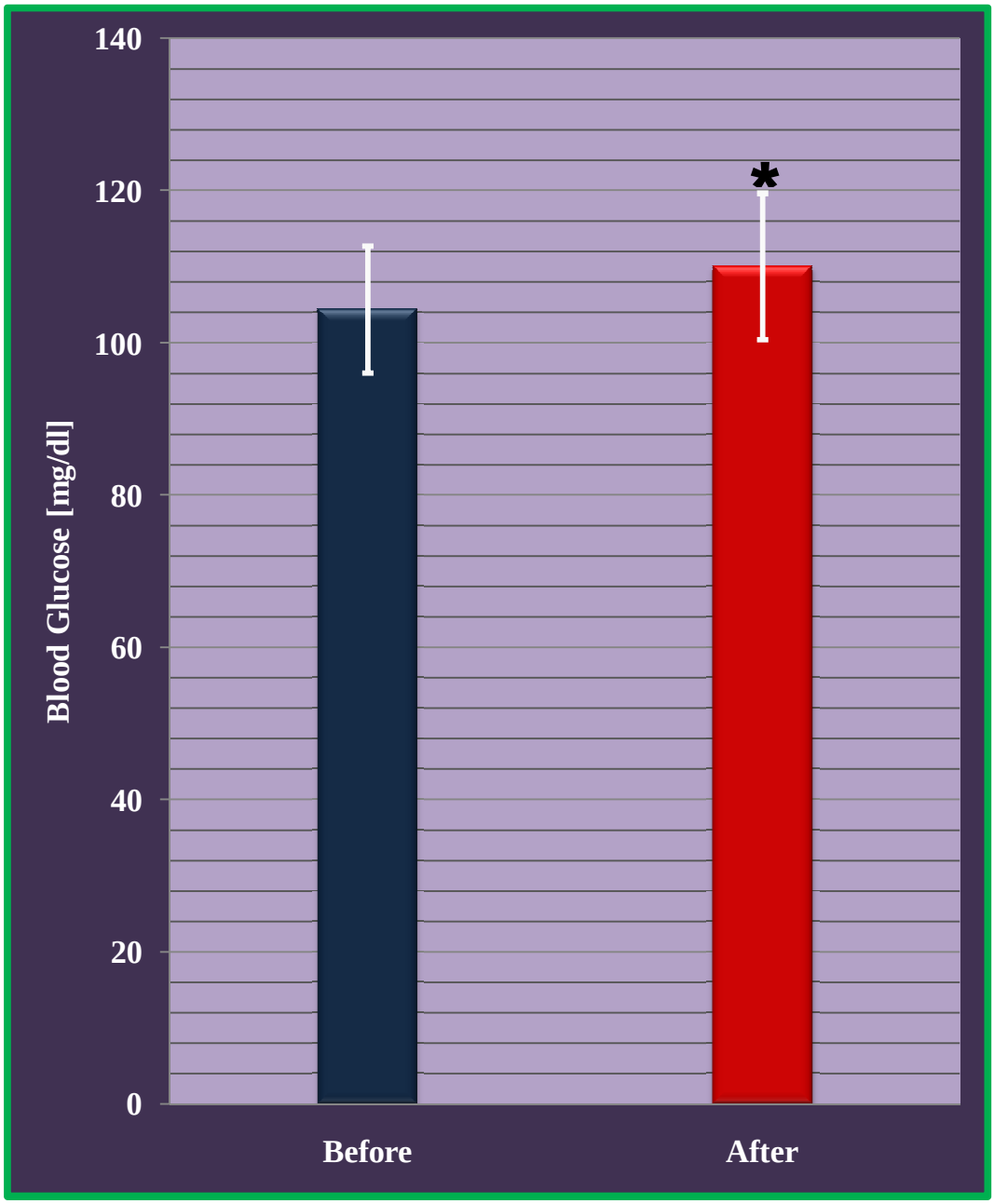
Data are represented as **Mean ± SD**

n = 36 [Female hypercholesteremic patients]

***P** < 0.0001 when compared to the values before the start of statins

Data are analyzed by using non-parametric Wilcoxon signed rank test

Figure 11. Bar diagram depicting the blood glucose [mg/dl] levels before and after 3 months of statins therapy in female hypercholesteremic patients



Data are represented as **Mean ± SD**

n = 36 [Female hypercholesteremic patients]

***P** < 0.004 when compared to the values before the start of statins therapy

Data are analyzed by using non-parametric Wilcoxon signed rank test

6. Discussion:

This study was minted to explore the effect of statin therapy on serum lipase and blood glucose levels in hyperlipidemic patients who have attended the outpatient department of the teaching tertiary care hospital in Kanyakumari District. This study included a total of 71 participants, each diagnosed to be a new case of hyperlipidemia and initiated on statin therapy.

Our study revealed that there was a statistically significant increase in both the levels of serum lipase and blood glucose after 3 months of statin therapy in hyperlipidemic patients.

In contrary to our study, various animal and human studies have shown that statins do not have negative impact on the pancreas.

Almeida et al.[109] conducted a study on male wistar rats have shown to decrease the cytokines due to inflammation and activation of neutrophils in lungs after inducing Acute Pancreatitis [AP] with cerulin or taurocholate. In a study conducted by Choi et al.[110] on cholecystokinin induced pancreatitis in male wistar rats which were pretreated with statin have shown that statins ameliorated the severity of pancreatitis.

Wei et al.[111] conducted an experimental study on male wistar rats to evaluate the effect of pravastatin on chronic pancreatitis. The above study showed, reduction of pancreatic inflammation. Mita et al.[112] conducted an open-label, crossover study on 24 patients which was have shown that pravastatin improved the pancreatic cell function.

The above studies were controversial to the results that have been obtained from our study which has shown that statin therapy has significantly increased the serum lipase levels. This result which is contentious to the above study may be due to the use of male wistar rats only, where as our study was conducted on humans of both sexes.

Prajapati et al.[92] reported a case of AP in a 58 year old male patient due to atorvastatin therapy which was confirmed by CT and elevated serum lipase level and the patient recovered within 10 days of stopping the drug. Chitanaboina et al.[10] and Singh et al.[11] reported cases of rosuvastatin induced AP which were confirmed by elevated serum lipase level and CT.

There were also case reports of acute pancreatitis due to simvastatin[12,14,91] and pravastatin[9,13], which were confirmed by clinical examination and laboratory investigations.[12,14,91]

Our study results were correlating with the various case reports[9-14,91,92] as discussed above, which has shown that statin therapy can lead to rise in serum lipase levels.

In this study, it was revealed that there was significant rise in blood glucose in the statin treated patients.

Shah et al.[113] in their study concluded that statin therapy leads to the risk of developing diabetes mellitus. A study conducted by Swerdlow et al.[114] revealed that patients having 3-hydroxy-3-methylglutaryl CoA reductase [HMGCR] gene with single nucleotide polymorphisms is associated with

increased body weight and risk of type 2 diabetes mellitus which was due to the insulin resistance with statin.

Aimen et al.[115] study proved that the possibility of developing new onset diabetes was increased with statin therapy and had a strong correlation with simvastatin, rosuvastatin and atorvastatin. Barylski et al.[116] in their study had shown that statins were useful in diabetic patients, but the risk of new onset diabetes was increased that overweighed the cardiovascular benefit. Dasgupta[117] in their study had concluded that there was a significant risk of developing new-onset diabetes.

Dormuth et al.[118] done a study on patients who were on statin therapy for secondary prevention of cardiovascular disease had concluded that use of statin having higher potency was moderately associated with a risk of developing new-onset diabetes.

Macedo et al.[119] concluded in their study that the use of statin had an association with a higher risk of developing type 2 DM and the risk was relatively higher in non hypertensive people. Kanda et al.[120] in his study on male Sprague Dawley rats had shown that atorvastatin significantly increased blood glucose level in oral glucose tolerance test [OGTT].

Our study results also have revealed that blood glucose level was significantly increased which was similar to the studies[113-120] conducted by various researchers around the world.

Satoh et al.[121] in his study on GK rats concluded that statin administration did neither improved the diabetes nor exaggerated the oral glucose tolerance test.

Otani et al.[122] conducted a study on rats and concluded that pravastatin therapy for longer duration had improved the status of diabetes mellitus and pancreatic fibrosis which was due to anti-oxidative and anti-fibrotic properties. This also concluded that stopping the administration of pravastatin had shown to revoke the beneficial effects and expedite DM and fibrosis of pancreas.

Marchand et al.[123] study result showed that the mass of the β-cell has expanded and raised the plasticity of islets in atorvastatin treated rats. Chen et al.[124] concluded in their study done on obese C57BL/6J mice that treatment with atorvastatin had increased the proliferation of pancreas and decreased the endoplasmic-reticulum stress on pancreas.

Studies conducted by various researchers[121-124] were not correlating with the results obtained from our current study. This could be due to the variation in the pharmacodynamic response of atorvastatin or rosuvastatin between animals and humans. Further, the participants in this study were on either atorvastatin or rosuvastatin, the effect of other members of statin group could not be evaluated.

United States Food and Drug Administration [USFDA] in 2012 stated the safety profile of statin that it can increase glycosylated haemoglobin and fasting blood glucose level.[125]

In this study, we assume that the negative impact of statin on pancreas leading to increased serum lipase levels would have been the cuase for raise in blood glucose levels.

The strength of this current study are; the study was registered in the Clinical Trial Registry-India, estimation of serum lipase and blood glucose levels were done using the same kit with same analyzer for all the study participants. Sample size was calculated using the appropriate formula by using the previous study on the prevalence of dyslipidemia[126] conducted in the southern part of Kerala which was located very close to the study site that reflects the status of hyperlipidemia. In this study, the treatment compliance was assessed by pill count method and factors that can cause AP are excluded.

The shortcomings of this current study were, other investigation tools like CT, USG and estimation of serum amylase were not done. Hence further study has to be considered on large population to extract a valid data about the effect of statin therapy on pancreas.

7. Conclusion:

Atorvastatin or rosuvastatin therapy for 3 months in newly diagnosed hyperlipidemic patients has significantly increased the serum lipase and blood glucose levels.

8. Summary:

Hyperlipidemia is due to an increase in the levels of various lipoproteins namely cholesterol, triglycerides, low density lipoproteins, high density lipoproteins and very low density lipoproteins and is an important factor that can lead to non-communicable disease like coronary artery disease and cerebrovascular disease in India.[1] The prevalence of dyslipidemia was 37.5% in the age group of 15-64 years and 62% in young males working in industry.[2] Hence it is necessary to screen for hyperlipidemia above the age of 20 years.[28]

Statins are found to be the most effective and well tolerated drug to reduce the lipid levels.[6,7] These drugs act by inhibiting the formation of mevalonate from 3 Hydroxy-3-methyl glutaryl Coenzyme A.[6,7] Various members of this group of drugs include atorvastatin, rosuvastatin, fluvastatin, lovastatin, pitavastatin, simvastatin and pravastatin.[6-8] Except atorvastatin and rosuvastatin all other statins should be administered at night time due to the shorter duration of action.[6]

Acute pancreatitis [AP] is an inflammatory condition of the pancreatic cell which clinically presents with abdominal pain, vomiting and fever and with the annual prevalence of 13-45/100000 persons.[85,87,93,94] The mortality is around 2-9% with AP.[88] The commonest causes of AP include gall stones, alcohol, endoscopic retrograde Colangiopancreatography, hypertriglyceridemia and drugs.[89] Apart from theses causes, various case

reports states that statin also could cause AP.[9-14,91,92] Estimation of serum lipase, amylase, blood glucose levels and radiological investigations plays a major role in diagnosing AP.[95]

Pancreatitis is found to be the cause for hyperglycemia and elevation of serum lipase and amylase by 3 fold than the normal levels are the diagnostic criteria for AP.[75,85]

There were no studies done in this part of Tamil Nadu to prove that statin therapy can increase serum lipase and blood glucose levels. Hence this study was designed to know the effect of statin therapy on serum lipase and blood glucose levels in hyperlipidemic patients who visit the teaching tertiary care hospital in Kanyakumari District [Tamil Nadu].

This was a prospective study, which enrolled 71 study participants, newly diagnosed as hyperlipidemia. Among the study participants, 35 were males and 36 were females. After getting the approval from Institutional Human Ethics Committee [IHEC], written informed consent was obtained and the patients were enrolled into the study. Baseline serum lipase and blood glucose levels were estimated in them and statin therapy was initiated. Study participants were advised to come for follow up after 3 months and serum lipase and blood glucose levels were estimated.

In this study, there was statistically significant rise in both serum lipase and blood glucose levels in participants after 3 months of statin therapy when compared to their baseline values. This study also showed that there

was significant increase in the serum lipase and blood glucose levels in female participants after 3 months of statin therapy, when compared to their baseline values. However, there was elevation in the levels of serum lipase and blood glucose in male study participants after 3 months of statin therapy, when compared to their baseline values, but it was found to be statistically insignificant.

These study findings were comparable to the previous studies reported in the literature.[9-14,91,92] However the results obtained in this study was controversial to some of the findings obtained from other studies.[109-112,122-124] This study excluded the study participants with factors which predisposed to the elevation of serum lipase and blood glucose levels. The pitfall of this study is the lack of CT scanning to confirm the diagnosis of acute pancreatitis.

The study concludes that there is a significant increase in the serum lipase and blood glucose levels after 3 months of statin therapy in hyperlipidemic patients. However, further long term clinical trials with larger sample size will be helpful to explore the effect of statin therapy on pancreas.

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Institutional Human Ethics Committee

Registered under CDSCO with Reg No. ECR/446/Inst/TN/2013

Ref. No. SMIMS/IHEC/2013/C/13 **Date: 27th December 2013**

Certificate

This is to certify that the Research Protocol Ref. No. **SMIMS/IHEC/2013/C/13,** entitled "Effect of Statin Therapy on Serum Lipase and Blood Glucose Levels in Patients with Hyperlipidemia" submitted by Dr. Prathab Asir A, Postgraduate of Department of Pharmacology, SMIMS has been approved by the Institutional Human Ethics Committee at its meeting held on 19th of December 2013.

[This Institutional Human Ethics Committee is organized and operates according to the requirements of ICH-GCP/GLP guidelines and requirements of the Amended Schedule-Y of Drugs and Cosmetics Act, 1940 and Rules 1945 of Government of India.]

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES ★ KULASEKHARAM ★
IHEC
Date: 27/12/2013

Dr. Rema Menon. N
Member Secretary
Institutional Human Ethics Committee
Professor of Pharmacology and HOD
SMIMS, Kulasekharam [K.K District]
Tamil Nadu -629161

CONSENT FORM

PART 1 OF 2

INFORMATION FOR PARTICIPANTS OF THE STUDY

Dear Participants,

We welcome you and thank you for your keen interest in participation in this research project. Before you participate in this study, it is important for you to understand why this research is being carried out. This form will provide you all the relevant details of this research. It will explain the nature, the purpose, the benefits, the risk, the discomforts, the precautions and the information about how this project will be carried out. It is important that you read and understand the contents of the form carefully. This form may contain certain scientific terms and hence, if you have any doubts or if you want more information, you are free to ask the study personnel or the contact person mentioned below before you give your consent and also at any time during the entire course of the project.

1. Name of the Principal Investigator : Dr. Prathab Asir. A,
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4. Institute : Sree Mookambika Institute of Medical Sciences, Kulasekaram, Kanyakumari District,TamilNadu.

5. Title of the study :

Effect of Statin Therapy on Serum Lipase and Blood Glucose Levels in Patients with Hyperlipidemia.

6. Background Information :

Abnormality in the lipid metabolism and plasma lipoproteins causes hyperlipidemia and statins are the commonly prescribed drugs for the treatment of hyperlipidemia. Even though no adverse effect of acute pancreatitis due to statin therapy have been mentioned in standard textbooks, there are few case reports of acute pancreatitis with statin therapy. Serum lipase and blood glucose levels are the diagnostic tools in acute pancreatitis.

7. Aims and objectives :

To study the Effect of Statin Therapy on Serum Lipase and Blood Glucose Levels in Patients with Hyperlipidemia.

8. Scientific justification of the study :

As there were case reports on statin induced acute pancreatitis and no standard textbooks states acute pancreatitis as an adverse effect of statins therapy, which is a frequently prescribed drug for the management of hyperlipidemia. It is necessary to do a study on Serum lipase and blood glucose levels for the patient on statin therapy.

9. Procedure for the study :

Under strict aseptic precautions 3 ml of venous blood will be collected from you in a plain sterile test tube for lipid profile estimation. If you are diagnosed to be a hyperlipidemic, written informed consent will be obtained from you and then evaluated for serum lipase and blood glucose levels before statin therapy. Then you will be advised to take statin for the management of hyperlipidemia. After the period of 3 months you will be advised to review at the General Medicine OPD for evaluation of lipid profile,

serum lipase and blood glucose levels with 3 ml of blood and all the readings will be noted. You will not have to spend additional expenses for the study related investigations.

10. Expected risk for the participants :

i. Risk of allergic reaction to drug.
ii. Pain at the site of drawing the blood.
iii. Thrombophlebitis – very rare.
iv. Hepatotoxicity.
v. Myalgia.
vi. Headache.

11. Expected benefits of research for the participants :

You may not have any personal gain because of this study but it may be beneficial to the society and medical fraternity in the future.

12. Maintenance of Confidentiality : Yes.

13. Why have I been chosen to be in this study : Hyperlipidemia.

14. How many people will be in the study : 71

15. Agreement of compensation to the participants :

Compensated by principal investigator as per the suggestions of IHEC.

16. Anticipated prorated payment, if any, to the participant's in the study : No.

17. Can I withdraw from the study at any time during the study period? : Yes.

18. If there is any new findings/ information, would I be informed? : Yes.

19. Expected duration of the participant's participation in the study : 3 months.

20. Any other pertinent information : No.

21. Whom do I contact for further information?

<u>For any study related queries, you are free to contact</u>

Dr. Prathab Asir. A,
I year Post Graduate, Department of Pharmacology,
Sree Mookambika Institute of Medical Sciences,
Kulasekharam – 629 161,
Mobile No. : +91-9443490137
Email ID : asirprathap@yahoo.com

Place : Kulasekharam, *Signature of the Principal Investigator*

Date :

Signature of the Participant

CONSENT FORM

PART 2 OF 2

PARTICIPANTS CONSENT FORM

The details of the study have been explained to me in writing. I am aware that the results of the study may not be directly beneficial to me but it will help in the advancement of medical sciences. I confirm that I have understood the study and had the opportunity to ask questions. I understand that my participation in the study is voluntary and that I am free to withdraw from the study at any time, without giving any reason and that it will not interfere with the normal course of treatment. I agree to make use of any data or results that arise from this study provided such a use is only for scientific purpose(s). I have been given an information sheet giving details of the study. I fully consent to participate in the study with the title "Effect of Statin Therapy on Serum Lipase and Blood Glucose Levels in Patients with Hyperlipidemia".

Serial No. : O.P. No. :

Name of the Participant :

Address of the Participant ;

Contact number of the participant :

Signature/ Thumb impression of the participant

Witnesses :

1.

2.

Place :Kulasekharam

Date :

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES

Kulasekharam, Kanyakumari District, Tamil Nadu, India – 629161

Department of Pharmacology

Title of the Study : Effect of Statin Therapy on Serum Lipase and Blood Glucose Levels in Patients with Hyperlipidemia

CASE RECORD FORM

Subject number: O.P. No. : I.P.No.: Date:

Name:

Age: Sex: M / F

Ht.: Wt.: BMI:

Occupation:

Address with contact number:

Diagnosis:

Family History: Diabetes Mellitus/ Hypertension/ Hyperlipidemia

Any other concurrent medication used? Yes / No. (If yes then details)

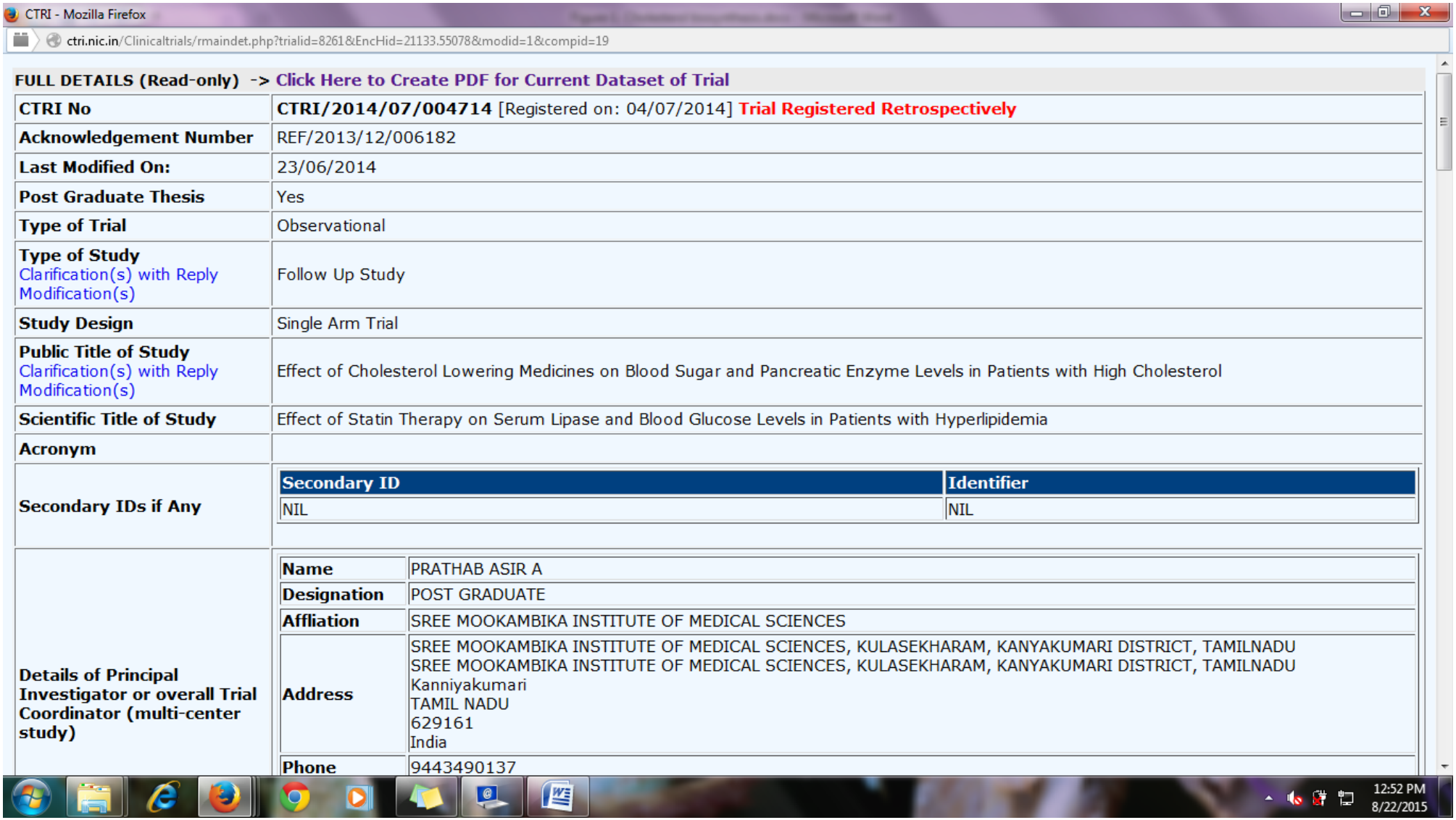
Prescription details

S.No	Non-proprietary name	Brand name	Company name	Form	Dose	Route	Frequency

Parameters:

Statin therapy	Date of visit	Serum Lipase (U/L)	B. Glucose mg/dl	TCH mg/dl	TGL mg/dl	LDL mg/dl	HDL mg/dl	VLDL mg/dl
Before								
After								

Signature of the Principal Investigator



FULL DETAILS (Read-only) -> Click Here to Create PDF for Current Dataset of Trial

CTRI No	**CTRI/2014/07/004714** [Registered on: 04/07/2014] **Trial Registered Retrospectively**
Acknowledgement Number	REF/2013/12/006182
Last Modified On:	23/06/2014
Post Graduate Thesis	Yes
Type of Trial	Observational
Type of Study Clarification(s) with Reply Modification(s)	Follow Up Study
Study Design	Single Arm Trial
Public Title of Study Clarification(s) with Reply Modification(s)	Effect of Cholesterol Lowering Medicines on Blood Sugar and Pancreatic Enzyme Levels in Patients with High Cholesterol
Scientific Title of Study	Effect of Statin Therapy on Serum Lipase and Blood Glucose Levels in Patients with Hyperlipidemia
Acronym	

Secondary IDs if Any

Secondary ID	Identifier
NIL	NIL

Details of Principal Investigator or overall Trial Coordinator (multi-center study)

Name	PRATHAB ASIR A
Designation	POST GRADUATE
Affliation	SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES
Address	SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES, KULASEKHARAM, KANYAKUMARI DISTRICT, TAMILNADU SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES, KULASEKHARAM, KANYAKUMARI DISTRICT, TAMILNADU Kanniyakumari TAMIL NADU 629161 India
Phone	9443490137

Image 4. Kit used for serum lipase estimation [Biosystems S.A., Spain]

Image 5. Brochure of serum lipase provided by the kit manufacturer

[Biosystems Reagents and Instruments]

COD 11793 1 x 60 mL
STORE AT 2-8ºC
Reagents for measurement of lipase concentration Only for *in vitro* use in the clinical laboratory

LIPASE

BioSystems REAGENTS & INSTRUMENTS

CE

LIPASE
COLOR

PRINCIPLE OF THE METHOD

Lipase catalyzes the hydrolysis of the chromogenic substrate 1,2-O-dialuryl-rac-glycerol-3-glutaric acid-(6'-methylresorufin)-ester to 1,2-O-dialuryl-rac-glycerol and an unstable intermediate, glutaric acid-(6'-methylresorufin)-ester. This descomposes spontaneously in alkaline solution to form glutaric acid and methylresorufin. The catalytic concentration is determined from the rate of the red dye formation measured at 580 nm[1].

1,2-O-dialuryl-rac-glycerol-3-glutaric acid-(6'-methylresorufin)-ester $\xrightarrow{\text{lipase}}$ 1,2-O-dialuryl-rac-glycerol + acid-(6'-methylresorufin)-ester

acid-(6'-methylresorufin)-ester $\xrightarrow{H_2O}$ glutaric acid + methylresorufin

CONTENTS AND COMPOSITION

A. Reagent: 1 x 50 mL. Tris buffer 40 mmol/L, colipase ≥ 1 mg/L, deoxycholate ≥ 1.8 mmol/L, taurodesoxycholate ≥ 7.0 mmol/L, pH 8.3.

B. Reagent: 1 x 10 mL. Tartrate buffer 15 mmol/L, 1,2-O-dialuryl-rac-glycerol-3-glutaric acid-(6'-methylresorufin)-ester ≥ 0.7 mmol/L, calcium ions ≥ 1 mmol/L, pH 4.0.

S. Lipase Standard: 1 for 1 mL. Human lipase in human serum matrix. Concentration is given on the label.

Components from human origin have been tested and found to be negative for the presence of antibodies anti-HIV and anti-HCV, as well as for Hbs antigen. However, they should be handled cautiously as potentially infectious.

STORAGE

Store at 2-8ºC.

Reagents and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: RA, presence of particulate material, turbidity. RB, is a turbid orange-colored microemulsion, discard if turning red. In some storage conditions (i.e. storage at a temperature lower than the one indicated) a precipitate may appear in the vial that will not influence the reagent performance, however, it is recommended to resuspend the product with a slight rotation of the vial before carrying out the analysis.

– Standard: Presence of moisture.

REAGENT PREPARATION

Reagents are provided ready to use.

Lipase Standard: Reconstitute with 1.00 mL of distilled water. Stable for 7 days at 2-8ºC or for 3 months at –18ºC when frozen in aliquots.

ADDITIONAL EQUIPMENT

– Thermostatic water bath at 37ºC.

– Analyzer, spectrophotometer or photometer with cell holder thermostatable at 37ºC and able to read at 580 ± 20 nm.

SAMPLES

Serum or sodium, lithium or ammonium heparin plasma collected by standard procedures.

Lipase in the sample is stable for 7 days at 2-8ºC.

PROCEDURE

1. Bring the Reagents and the instrument to 37ºC.
2. Pipette into a cuvette: (Note 1)

Reagent A	1000 µL
Serum / Standard (S)	10 µL

3. Mix and insert the cuvette into the instrument. Start the stopwatch. After 1-3 minute, add:

Reagent B	200 µL

4. Mix.
5. After 1 minute, record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
6. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (ΔA/min).

CALCULATIONS

The lipase concentration in the sample is calculated using the following general formula:

$$\frac{\Delta A/min_{\ Sample}}{\Delta A/min_{\ Standard}} \times C_{\ Standard} = U/L$$

REFERENCE VALUES

Serum: ≤ 38 U/L = ≤ 0.633 µkat/L

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit 5.0 U/L lipase = 0.083 µkat/L lipase

– Linearity limit: 250 U/L = 4.17 µkat/L lipase. For higher values dilute sample 1/2 with distilled water and repeat measurement.

– Repeatibility (within run):

Mean concentration	CV	n
119 U/L = 1.98 µkat/L	3.4 %	20
215 U/L = 3.58 µkat/L	2.8 %	20

– Reproducibility (run to run):

Mean concentration	CV	n
119 U/L = 1.98 µkat/L	4.5 %	25
215 U/L = 3.58 µkat/L	5.0 %	25

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

– Interferences: Lipemia (triglycerides < 30 g/L) and bilirubin (< 20 mg/dL) do not interfere. Hemoglobin (> 5.0 g/L) interfere. Other drugs and substances may interfere[2].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Lipases hydrolyzes glycerol esters of long-chain fatty acids. Although lipase can be secreted by other glands and mucosa, only pancreatic lipase is of interest in medical diagnosis. Therefore, lipase measurements on serum are used exclusively to investigate pancreatic disorders.

Serum lipase concentration increases after an attack of acute pancreatitis. In general, increases in amylase and lipase run in parallel course, but the elevation of lipase persists for a longer time. Elevations in serum lipase concentration may be also due to obstruction of the pancreatic duct by a calculus or by carcinoma, in acute and chronic renal disease as well as in treatments with opiates[3,4].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.

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M11793i-01

BioSystems S.A. Costa Brava, 30. 08030 Barcelona (Spain)

Quality System certified according to EN ISO 13485 and EN ISO 9001 standards

10/2009

Image 6. Instrument used for estimation of serum lipase [Beckman Coulter Chemistry analyzer AU 480]

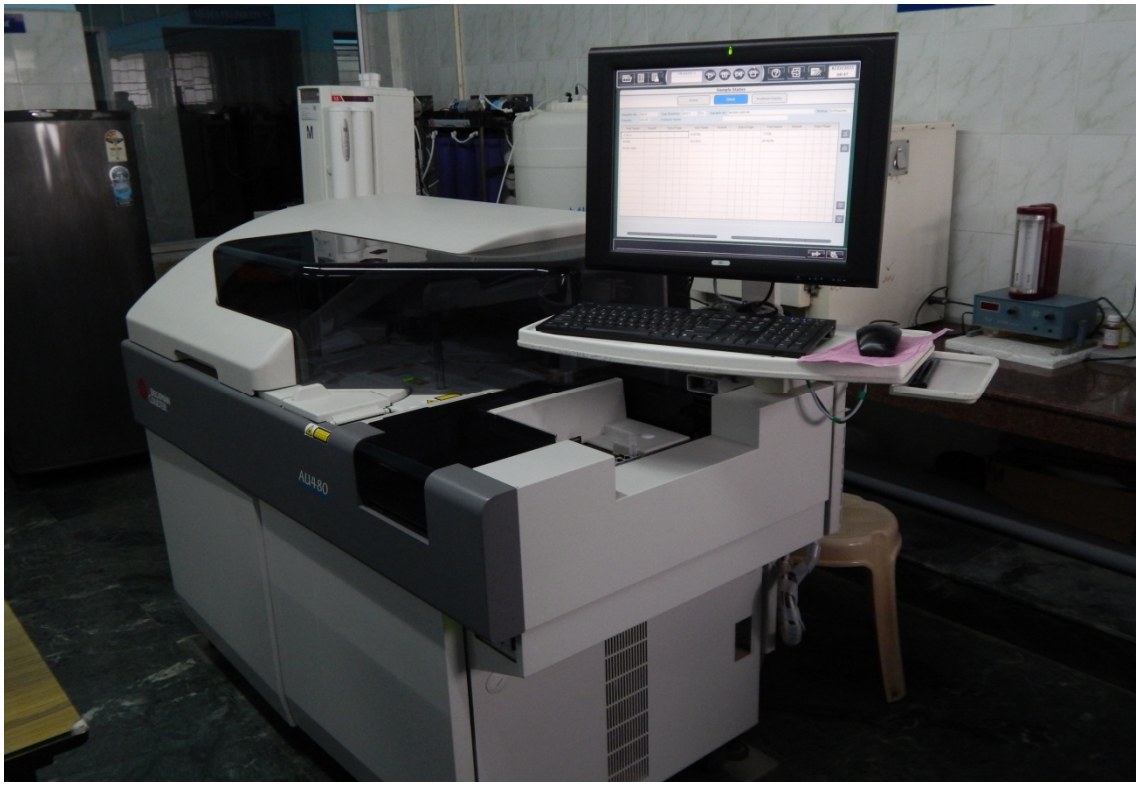
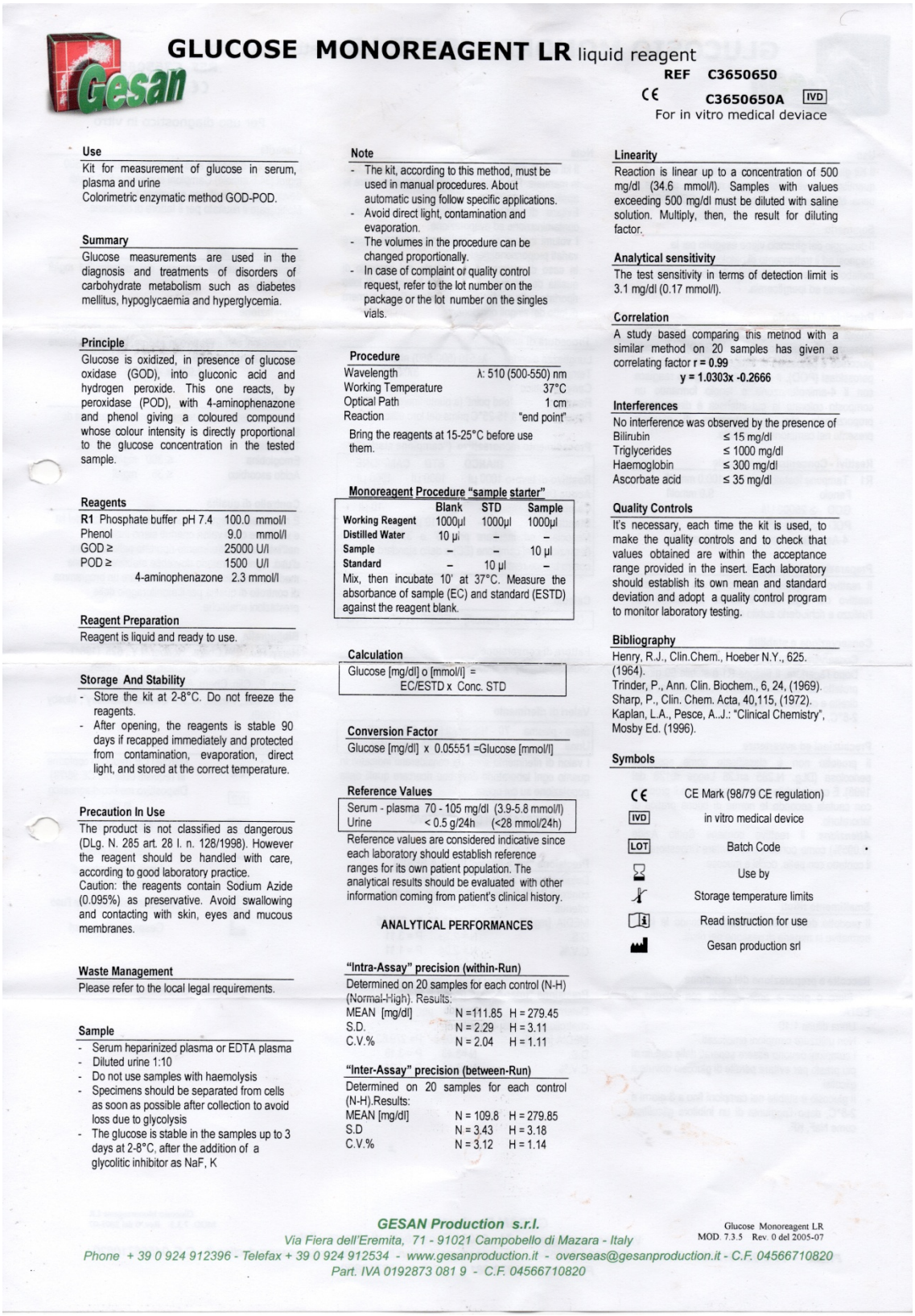


Image 7. Kit used for estimation of blood glucose [Glucose Monoreagent LR liquid reagent, Gesan production s.r.l. Italy.]

Image 8. Brochure of blood glucose monoreagent provided by the kit manufacturer [Gesan Production s.r.l]



GLUCOSE MONOREAGENT LR liquid reagent

REF C3650650
CE C3650650A IVD
For in vitro medical deviace

Use
Kit for measurement of glucose in serum, plasma and urine
Colorimetric enzymatic method GOD-POD.

Summary
Glucose measurements are used in the diagnosis and treatments of disorders of carbohydrate metabolism such as diabetes mellitus, hypoglycaemia and hyperglycemia.

Principle
Glucose is oxidized, in presence of glucose oxidase (GOD), into gluconic acid and hydrogen peroxide. This one reacts, by peroxidase (POD), with 4-aminophenazone and phenol giving a coloured compound whose colour intensity is directly proportional to the glucose concentration in the tested sample.

Reagents

R1 Phosphate buffer pH 7.4	100.0	mmol/l
Phenol	9.0	mmol/l
GOD ≥	25000	U/l
POD ≥	1500	U/l
4-aminophenazone	2.3	mmol/l

Reagent Preparation
Reagent is liquid and ready to use.

Storage And Stability
- Store the kit at 2-8°C. Do not freeze the reagents.
- After opening, the reagents is stable 90 days if recapped immediately and protected from contamination, evaporation, direct light, and stored at the correct temperature.

Precaution In Use
The product is not classified as dangerous (DLg. N. 285 art. 28 l. n. 128/1998). However the reagent should be handled with care, according to good laboratory practice.
Caution: the reagents contain Sodium Azide (0.095%) as preservative. Avoid swallowing and contacting with skin, eyes and mucous membranes.

Waste Management
Please refer to the local legal requirements.

Sample
- Serum heparinized plasma or EDTA plasma
- Diluted urine 1:10
- Do not use samples with haemolysis
- Specimens should be separated from cells as soon as possible after collection to avoid loss due to glycolysis
- The glucose is stable in the samples up to 3 days at 2-8°C, after the addition of a glycolitic inhibitor as NaF, K

Note
- The kit, according to this method, must be used in manual procedures. About automatic using follow specific applications.
- Avoid direct light, contamination and evaporation.
- The volumes in the procedure can be changed proportionally.
- In case of complaint or quality control request, refer to the lot number on the package or the lot number on the singles vials.

Procedure

Wavelength	λ: 510 (500-550) nm
Working Temperature	37°C
Optical Path	1 cm
Reaction	"end point"

Bring the reagents at 15-25°C before use them.

Monoreagent Procedure "sample starter"

	Blank	STD	Sample
Working Reagent	1000µl	1000µl	1000µl
Distilled Water	10 µl	–	–
Sample	–	–	10 µl
Standard	–	10 µl	–

Mix, then incubate 10' at 37°C. Measure the absorbance of sample (EC) and standard (ESTD) against the reagent blank.

Calculation
Glucose [mg/dl] o [mmol/l] =
EC/ESTD x Conc. STD

Conversion Factor
Glucose [mg/dl] x 0.05551 =Glucose [mmol/l]

Reference Values

Serum - plasma	70 - 105 mg/dl	(3.9-5.8 mmol/l)
Urine	< 0.5 g/24h	(<28 mmol/24h)

Reference values are considered indicative since each laboratory should establish reference ranges for its own patient population. The analytical results should be evaluated with other information coming from patient's clinical history.

ANALYTICAL PERFORMANCES

"Intra-Assay" precision (within-Run)
Determined on 20 samples for each control (N-H) (Normal-High). Results:

MEAN [mg/dl]	N =111.85	H = 279.45
S.D.	N = 2.29	H = 3.11
C.V.%	N = 2.04	H = 1.11

"Inter-Assay" precision (between-Run)
Determined on 20 samples for each control (N-H).Results:

MEAN [mg/dl]	N = 109.8	H = 279.85
S.D	N = 3.43	H = 3.18
C.V.%	N = 3.12	H = 1.14

Linearity
Reaction is linear up to a concentration of 500 mg/dl (34.6 mmol/l). Samples with values exceeding 500 mg/dl must be diluted with saline solution. Multiply, then, the result for diluting factor.

Analytical sensitivity
The test sensitivity in terms of detection limit is 3.1 mg/dl (0.17 mmol/l).

Correlation
A study based comparing this metnod with a similar method on 20 samples has given a correlating factor **r = 0.99**
y = 1.0303x -0.2666

Interferences
No interference was observed by the presence of

Bilirubin	≤ 15 mg/dl
Triglycerides	≤ 1000 mg/dl
Haemoglobin	≤ 300 mg/dl
Ascorbate acid	≤ 35 mg/dl

Quality Controls
It's necessary, each time the kit is used, to make the quality controls and to check that values obtained are within the acceptance range provided in the insert. Each laboratory should establish its own mean and standard deviation and adopt a quality control program to monitor laboratory testing.

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Kaplan, L.A., Pesce, A..J.: "Clinical Chemistry", Mosby Ed. (1996).

Symbols

CE	CE Mark (98/79 CE regulation)
IVD	in vitro medical device
LOT	Batch Code
	Use by
	Storage temperature limits
	Read instruction for use
	Gesan production srl

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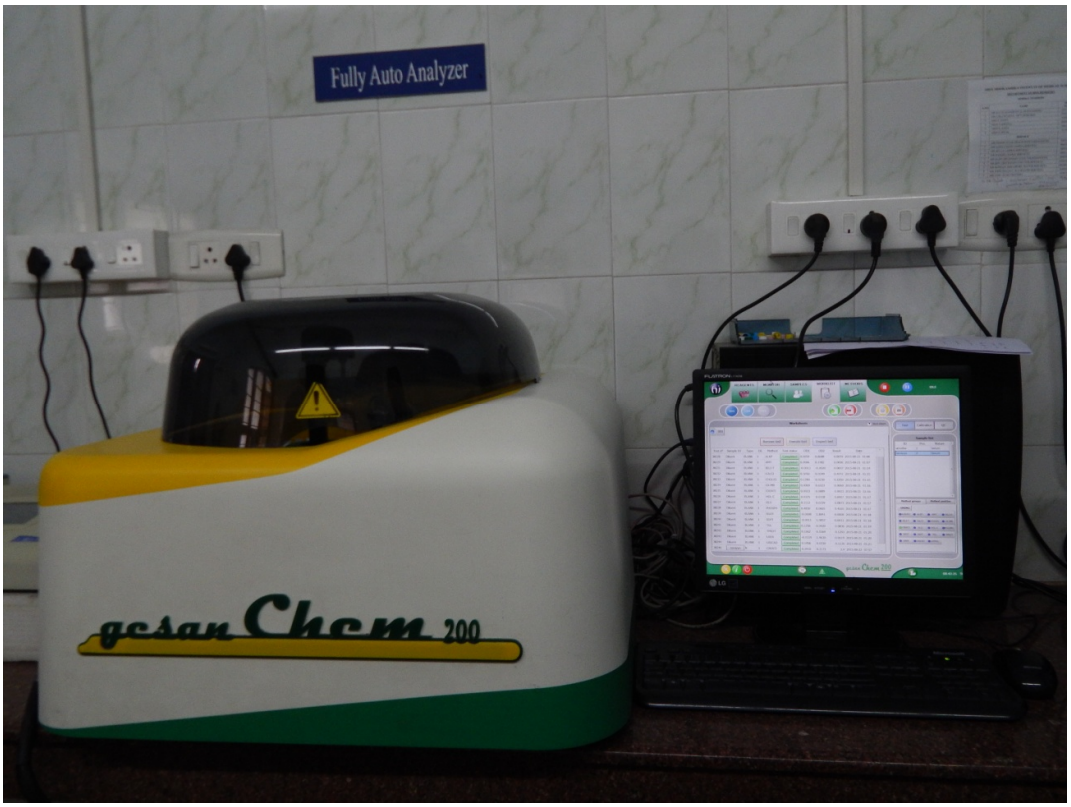
Glucose Monoreagent LR
MOD 7.3.5 Rev. 0 del 2005-07

Image 9. Instrument used for estimation of Blood glucose [Gesan Chem 200 clinical chemistry autoanalyzer]



ABBREVIATIONS

List of abbreviations	
%	Percentage
µl	Microlitre
ABG	Arterial blood gas
ADRs	Adverse drug reactions
ALT	Alanine aminotransferase
AMY	Amylase
AP	Acute pancreatitis
APACHE	Acute physiology and chronic health evaluation
APOE	Apolipoprotein E
ARM	Age related maculopathy
AST	Aspartate transaminase
ATP	Adenosine triphosphate
ATP III	Adult Treatment Panel III
BISAP	Bedside index of severity in acute pancreatitis
BMI	Body mass index
BPH	Benign prostatic hyperplasia
BSI	Blood stream infection
BUN	Blood urea nitrogen
CAD	Coronary artery disease
CETP	Cholesterol esterase transfer protein
CKD	Chronic kidney disease
CoA	Coenzyme A
CRP	C-Reactive Protein
CT	Computerized tomomgraphy
CTRI	Clinical Trial Registry - India
CTSI	Computerized tomomgraphy severity index
CVD	Cerebrovascular disease
CYP	Cytochrome P
Da	Dalton
DHA	Docosahexaenoic acid
DKA	Diabetic keto acidosis
DM	Diabetes mellitus
EPA	Eicosapentaenoic acid
ERCP	Endoscopic Retrograde Cholangio Pancreatography
gms	Grams
G-CSF	Granulocyte colony stimulating factor
GI	Gastrointestinal
H	Hour
HbA1C	Glycosylated hemoglobin
HDL	High-density lipoprotein

HES	Hydroxyethyl starch
HGF	Hepatocyte growth factor
HMG-CoA	3-Hydroxy-3-methyl glutaryl coenzyme A
HMGCR	3-Hydroxy-3-methyl glutaryl coenzyme A reductase
HOMA-IR	Homeostatic model assessment-insulin resistance
HONK	Hyper osmolar non ketotic coma
hs-CRP	High-sensitivity C-reactive protein
Ht	Height
ICAM	Inter cellular adhesion molecule
IHEC	Institutional Human Ethics Committee
IL	Interleukin
IPE	Icosapent ethyl
IV	Intravenously
kg	Kilogram
LCAT	Lecithin cholesterol acyl transferase
LDL	Low-density lipoproteins
LDLR	low-density lipoprotein receptor
LPL	Lipoprotein lipase
LPS	Lipase
MDCT	Multi detector computerized tomography
mg/dl	Milligram per decilitre
mins	Minutes
ml	Millilitre
MMP	Metalloproteinases
MPTP	1-methyl-4-phenyl-1,2,3,6-tetra hydropyridine
MRI	Magnetic resonance imaging
NADH	Nicotinamide dinucleotide hydride
NCEP	National Cholesterol Education Programme
NCMH	National Commission on Macroeconomics and Health
No.	Number
NS	Normal saline
NSAID	Non steroidal anti-inflammatory drug
OATP	Organic anion transporter
OATP1B1	Organic anion transporting polypeptide 1 B1
OCP	Oral contraceptive pill
OGTT	Oral glucose tolerance test
PAI-1	Plasminogen activator inhibitor-1
PAP	Pancreatitis associated protein
PLA2	Phospholipase A2
PVD	Peripheral vascular disease
RBS	Random blood sugar
RCT	Randomized clinical trial
rpm	Rotations per minute

SAP	Severe acute pancreatitis
SD	Standard deviation
SLE	Systemic lupus erythromatosis
SMIMS	Sree Mookambika Institute of Medical Sciences
SNP	Single nucleotide polymorphism
SREBPs	Sterol Regulatory Element-Binding Proteins
TCH	Total cholesterol
TGL	Triglycerides
TNF-α	Tumor necrosis factor alpha
TTP	Thrombocytopenic purpura
U/L	Unit per litre
USFDA	United States Food and Drug Administration
USG	Ultrasonography
VF	Ventricular Fibrillation
VIP	Vasoactive intestinal polypeptide
VLDL	Very low density lipoprotein
VT	Ventricular tachycardia
WBC	White blood cell
WHO-UMC	World Health Organization-Uppsala Monitoring Centre
Wt	Weight
α	Alpha
β	Beta
δ	Delta
µl	Micro litre